**DRAFT**

# Cruise Report

## *RRS Discovery* Cruise 243

**11th October to 22nd November 1999**

## *"Sensory Biology in the Deep-Sea: Anatomy, Physiology, and Molecular Biology"*

**Principal Scientist**
**Julian C. Partridge Ph.D.**

**2000**

**CONTENTS**

## 1. R.R.S. DISCOVERY CRUISE 243: PERSONNEL

| NO. | SURNAME | FORENAMES | RANK |
|---|---|---|---|
| 1 | PLUMLEY | Robin | MASTER |
| 2 | SARJEANT | Peter | C/O |
| 3 | SYKES | Syd | 2/O |
| 4 | OWOSO | Titus | 3/O |
| 5 | McDONALD | Bernie | C/E |
| 6 | ROYSTON | Jim | 2/E |
| 7 | HEALY | Tony | 3/E |
| 8 | SLATER | Gary | 3/E |
| 9 | STEWART | Dave | ETO |
| 10 | TREVASKIS | Mike | CPOD |
| 11 | LUCKHURST | Kevin | POD |
| 12 | ALLISON | Phil | SG1A |
| 13 | COOK | Stuart | SG1A |
| 14 | COOPER | Gerry | SG1A |
| 15 | DALE | John | SG1A |
| 16 | JOHNSON | Bob | SG1A |
| 17 | DICK | David | MM1A |
| 18 | STAITE | Eddie | SCM |
| 19 | HAUGHTON | John | CHEF |
| 20 | DUNCAN | Andy | M/STWD |
| 21 | LINK | Wally | STWD |
| 22 | LINK | Sheila | STWD |
| 23 | PARTRIDGE | Julian | SCI |
| 24 | ALLEN | Penny | SCI |
| 25 | BAGLEY | Phil | SCI |
| 26 | BOORMAN | Ben | SCI |
| 27 | BOWMAKER | Jim | SCI |
| 28 | EDGE | Dave | SCI |
| 29 | FRANK | Tammy | SCI |
| 30 | HENRIQUES | Camila | SCI |
| 31 | HERRING | Peter | SCI |
| 32 | HOWELL | Penny | SCI |
| 33 | HUNT | David | SCI |
| 34 | INCE | Rachel | SCI |
| 35 | MARSHALL | Justin | SCI |
| 36 | MERRETT | Nigel | SCI |
| 37 | NYHOLM | Spencer | SCI |
| 38 | PRIEDE | Monty | SCI |
| 39 | REES | Jean-Francois | SCI |
| 40 | SHALE | David | SCI |
| 41 | SHELTON | Peter | SCI |
| 42 | STEFANNI | Sergio | SCI |
| 43 | WAGNER | Jochen | SCI |
| 44 | WAY | Sue | SCI |
| 45 | WIDDER | Edie | SCI |
| 46 | DUNCAN | Paul | SCI/RVS |
| 47 | ROBERTS | Rhys | SCI/RVS |
| 48 | SMITH | Kevin | SCI/RVS |
| 49 | TAYLOR | Phil | SCI/RVS |
| 50 | YOUNG | Darren | SCI/RVS |

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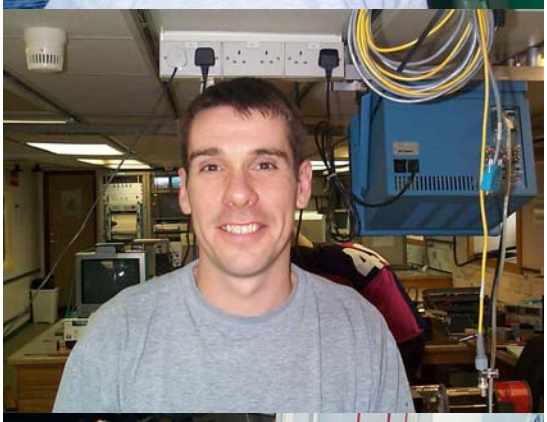

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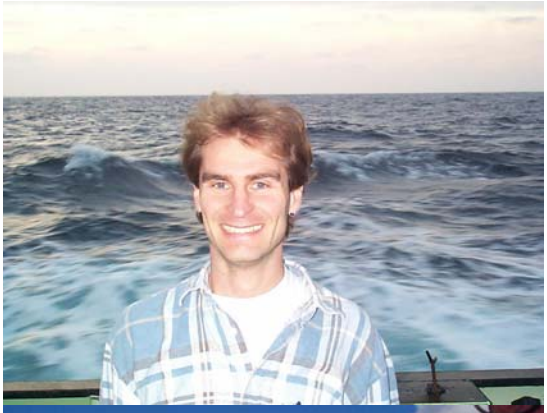

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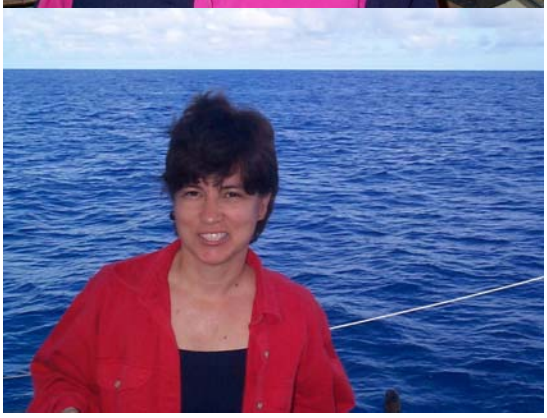


## 3. ITINERARY

Depart Southampton, UK, 11th October 1999
Arrive Cadiz, Spain, 22nd November 1999

## 4. OBJECTIVES

1. To collect biological samples for anatomical, physiological and molecular biological studies, by midwater and benthic trawling, from eutrophic and oligotrophic regions of the tropical East Atlantic
2. To deploy benthic landers for studies on fish mobility, as well as benthic and midwater bioluminescence.

## *5. OVERVIEW*

*RRS Discovery* cruise 243 had two principal objectives: to collect deep-sea animals for a variety of biological studies ranging from physiology to molecular biology, and to deploy benthic landers. The landers are described in Section 9.1, and the midwater and benthic trawling in Section 9.2. A complete list of the stations worked, with times, latitudes and longitudes, is given in Section 10, and a track chart for the whole cruise is shown in Section 11.

Trawling was conducted first and foremost in order to provide specimens required for work associated with NERC grant GR3/B1212 "Analysis of light-induced interactions in the deep-sea: bioluminescence and its relation to vision, reflectance and fluorescence" to Professor Peter Herring, Dr Julian Partridge, and Dr Peter Shelton. Of equal importance to the success of the cruise, however, was the provision of biological samples for a range of inter-related studies. Descriptions of these studies are given in Section 9.3.

Throughout the cruise a narrative (Section 6) was compiled to document, in diary format, the main scientific activities on board ship. It also records information about factors which affected work, such as the weather, and problems with equipment. The narrative is more or less a list of notes rather than proper prose, and was written during the cruise, documenting events as they were planned, as they unfolded, or after they had happened. In consequence, the tenses of verbs tend to vary in a haphazard way, for which I apologise.

During the cruise, the *Discovery* "Rough Log" of biological specimens was maintained by Professor Peter Herring. A précis of some of the information from the Rough Log is incorporated into the narrative as a record of some of the most common, and some of the most unusual, animals that were collected. This is necessarily a biased record. Nevertheless, it may be useful to others planning or conducting similar cruises to the areas worked during D243, particularly those targeting the pelagic macrofauna.

As the narrative shows, D243 was plagued by problems with the main winch. At one stage, early in the cruise, the severity of these problems made it likely that the cruise would have to be terminated prematurely, without any trawling having been undertaken. That this situation was reversed is due to the determination and hard work of the RVS technicians who were on board the ship. To them, Phil Taylor (RVS Technical Liaison Officer/TLO), Kevin Smith (RVS Mobilisation Officer/MO), Paul Duncan, and Rhys Roberts, we are much indebted: without their labours the cruise would not have succeeded in the way it ultimately did.

Inevitably, the "science time" of the cruise was affected by the winch problems (see Section 8) and was contributory to a decision not to work the slope of the African continent, which had been part of the original cruise directive. This was, however, to some extent compensated by the generally stable weather conditions in the work area. Although we encountered conditions more extreme than are indicated

in the ‘Africa Pilot’ for the region during October/November (anticipated average wind force 3), at no time was work stopped by poor weather. This in itself partly justifies the relatively long passage time to the work area. The main reason for working in the region, however, was the high diversity and abundance of midwater and benthic macrofauna in this region of upwelling and high surface water productivity. This, in combination with the trawling methods used, which included the use of the relatively large RMT25 net and a closing cod end on the RMT8 net, ensured that the requirements of the scientific personnel for specimens were well met.

The scientific complement of the cruise consisted of 23 people, ranging from graduate students to professors, from five countries and eleven institutions. In addition, the cruise also hosted a team from the BBC Natural History Unit, who were on board to film for the “The Blue Planet”, a television series about the seas which is due for release in 2001. That this diverse group (who are listed, with their contact details, in Section 2) worked so well together, and were steadfastly cheerful firstly in the face of the winch problems, and later in the face of the relentlessness of trawling and catch processing, is very much to their credit. As Principal Scientist on D243 I am extremely grateful for their hard work and support in the run-up to the cruise, during the time at sea, and in its aftermath. In particular I would like to single out for thanks Ben Boorman and Nigel Merrett who, as scientific day and night watch leaders, ensured that the fishing and the supply of specimens continued without a break.

D243 was also notable for one other event: it was Peter Herring’ last cruise before his retirement from the Southampton Oceanography Centre. Without a doubt, none of the participants on D243 would have been there but for Peter, such has been his impact on ocean going biology. Indeed, the format of D243 very much follows the successful formula developed by him on numerous previous cruises, including some to the Cape Verde region of the West African upwelling. If a cruise report can be dedicated, this is dedicated to him.

Finally, I would like to thank the Captain and crew of *RRS Discovery* for their professionalism and cheerfulness which ensured a very memorable, enjoyable, and highly successful cruise.

**Julian C. Partridge.**
**Principal Scientist, *RRS Discovery* cruise 243.**

6. Narrative

**Monday October 11th 1999**

All personnel to sail with ship arrived by afternoon at Southampton Oceanography Centre, Empress Dock, Southampton. Professors David Hunt and Jim Bowmaker (Institute of Ophthalmology), dropped off their gear and departed. Much of the day spent loading scientific gear and stowing it for sea. Safety briefing at 1500 by the Master.

During day the Principal Scientist (PSO) and Peter Herring (PJH) were interviewed by BBC South for television. PSO also interviewed by Western Daily Press (Bristol), and by Radio Bristol.

The bonded truck delivering gear belonging to Drs Edie Widder and Tammy Frank to *Discovery* eventually arrived at 2200 via Amber Freight, and three pallets, wrapped in cling-film, were broken down and hand loaded onto the ship with help from Chief Officer, boatswain and crew. Several boxes clearly damaged, and a VDU monitor destroyed in transit.

Organisation of the day would not have been possible without a mobile 'phone!

**Tuesday October 12th**

Screening of BBC TV interviews in morning. PJH interviewed live by Radio Solent; PSO by local FM radio station.

Problems with engine resolved by midday, but delayed sailing from 1000 to 1300.

1327 Let go Empress dock (waved off by SOC staff); 1340 Cleared the dock; 1503 Pilot off the ship; 1515 Fire and boat drill, followed by compass swing in the Solent.

Afternoon and evening spent continuing to organise lab space and set up equipment.

**Wednesday October 13th**

0900 Meeting with the Captain, boatswain, MO, PJH, Professor Monty Priede (IGP), and C/O to discuss initial work plans and related issues. At this stage:

(i) Permission to work has only been received from Spain, and we are thus not able to work off Senegal or other EEZs as planned. Chart work identifies a small corridor beyond the 200 mile limits and close to the preferred area, bottom depth 3-4km, and a box 20-22N, 21-25W in the NW corner of the proposed work area. However, most of the planned work area is out of bounds at present. Master to request RVS to try to obtain permission once again.

(ii) A deep water area off Spain (44ºN 11ºW) identified as suitable for a winch test, which will be combined with a lander wire deployment to test acoustic releases and ISIT lander electronics. This planned for ca. 1000 October 13th. We have about 6 hours in hand for this exercise, time being made up by good weather conditions.

(iii) South of Tenerife, plan buoyancy check on ISIT lander. Deploy lander with excess buoyancy, count floats on surface, recover lander. This also as a test on deployment and recovery methods.

(iv) Work plan for working area agreed. This will be a 24 hour schedule of RMT 1+8CCE deployments, interleaved with a morning recovery of landers, and afternoon/evening deployment of landers. Boatswain agreed to crewing of 2 ABs at all times, although this will not allow him to do much maintenance. 2 ABs and RVS technicians, as well as scientific personnel will be needed for both lander and network.

A watch system for scientific staff was promulgated to be initiated when in work area, with Ben Boorman i/c day watch and Nigel Merrett i/c night watch.

1130 Verbal permissions to work in Mauritania and Cape Verde waters obtained following telephone call to RVS from Master. Paperwork to follow. Nothing yet from Senegal. Chris Adams, RVS, chasing.

1630 No objections from submariners or Spanish authorities, so on course for winch test with ISIT lander on wire in 4.6km depth tomorrow.

**Thursday 14th October 1999**

Clearance given by Spain, Mauritania and Cape Verdes now confirmed in writing. Master plotted area of work and most of original work area now possible. With additional permission from Senegal, work further south would also be possible.

1030 Hove to for winch test and test of lander releases at station above. Winch gave problems before gear deployed. Deployment delayed until after lunch.

1248. **Station D243#1** ISIT lander deployment on wire as winch and ISIT test. Lander rigged to test ISIT ballast releases, ISIT acoustics, ISIT camera control, AUDOS releases, and to run a timed on/off video sequence. PES deployed also. [Note: Station numbers not in *Discovery* sequence at this stage as this information was not available on ship via bridge or computer operator. Hence unique sequence started with each deployment having a new number until we can find the *Discovery*.].

No winch wire out or veer/haul information available on Seametrix monitors in lab/bridge, so we are dependent on winch counters (in winch room only) and/or Aberdeen over-the-side transducer. These are not in agreement (e.g. Aberdeen gear indicates ISIT at 232m when winch room indicates 70m cable out!). ISIT camera turned on/off during deployment and winch stopped when ISIT camera recording on. [Note: Winch counter, mechanical or electrical, is needed for trawling. Without it we depend on the net monitor to give a depth of the net. This, however, gives an underestimation of wire out and hence a safe estimate on retrieval.]

Winch hydraulics working adequately (1500) with no over heating and veer haul rate increased to *ca*. 40m/min (Aberdeen estimate) to get work done. We will have used up most of time in hand by the time of retrieval and will need to get underway. Gear deployed to 3000m and retrieved by ca 1700. All releases worked. After recovery ship set course for Canaries, keeping outside territorial waters of Spain etc. Master calculates spare time for buoyancy test tomorrow morning.

Video from ISIT camera shows camera able to see good image at 230m depth. Much bioluminescence at 500m including splats by jellies on splat screen. Much less at 900m.

**Friday 15th Oct 1999**

Onto GMT at 0200. Poor weather (rain and sea state 4-5) suggest abandonment of buoyancy test until later. Continue steam to Canaries. By 1700 weather improving, wind abating and sea state settling.

Continued to set up laboratory space. Rachel Ince (PGRA on NERC grant) obtains data from *Crangon crangon,* brought with her from Bristol, having been collected in Scotland by Millport Marine Station. They have had a long journey.

Information to PJH from SOC enables station numbers to be revised to fit into Discovery station numbering system. First station of this cruise, formerly **D243#**1, thus revised to **13630#1.**

**Saturday 16th Oct 1999**

Wind abated and weather improved. Position at 0800 and estimated steaming time to Tenerife suggest time in hand for buoyancy test on ISIT lander. This scheduled for 1030 when still well outside national waters of Madeira or mainland Portugal. Since this is a test deployment, not designed to yield scientific data, no station number is given.

1030 Test deployment of lander at 36º 50.6′N 14º 30.9′W for training of crew in launch and recovery methods, and test of buoyancy of lander. Recovery at 1150. Deployment and recovery methods worked well, with only the minor technical hitch of

the deck winch scrolling system not traversing properly. Solved by transfer to other deck winch. Lander slightly too buoyant, by some 25kg.

**Sunday 17th Oct 1999**

Excellent weather during passage to Tenerife: 4/10 cloud , blue water, blue sky, wind force 1.

Phil Bagley investigated winch wire out problem with RVS SEG technicians and found wire-out sensor operating correctly.

Approaching Tenerife, receive messages from Jochen Wagner to the effect that he had not been met at the airport and had had no contact with the agent. Master telexes agent and PSO 'phones JW giving mutual contact information. Hope that this resolves itself.

**Monday 18th Oct 1999**

0800 Approaching Tenerife; 0955 08º 27.7′N 16º 09.2 W Launch BEATRIZ-1 alongside with 11 passengers for transfer – fortunately Jochen has been found (!); 1026 Launch away; 1100 Safety briefing for new arrivals from C/O; 1400 Science briefing; 1615 Emergency muster and boat drill, with additional exercises. Four first-aid qualified members of science party visited hospital facilities. Peter Shelton represented scientific party to explain to Bridge planned action in the even of a chemical spill.

Steam to proposed work area at 26º 42.5′ N 18º 09.5′ W for RMT scheduled for RMT 1+8 CCE test deployment.

Planned calibration of ship's log deferred in order for RVS technicians to work on winch as a priority.

At this stage it was found that Penny Allen's personal gear, including Digital Beta tapes (approx. 40% of the BBC stock on board), and a high resolution video monitor was not on the ship. Tracing back found the items had been delivered to the SOC but had not been taken to the ship as no-one was informed of the delivery. Arrangements made for DHL to get gear to Tenerife ASAP. Feedback is that delivery will be Wednesday 20th or Thursday 21st October which is likely to be too late unless we are held up by the winch work.

**Tuesday 19th Oct 1999**

RMT deployment method agreed with Ben Boorman and PJH and with Bridge.

0905 Precision Echo Sounder (PES) wire faired (delaying proceedings) and PES deployed

0920 RMT 1+8 CCE deployed to test system and to train crew in launch/recovery. Deployed by Ben Boorman and 2 ABs. Station number **13631 #1**. Planned fishing depth 700 m. Deployment slow but without problems. Net acoustic monitor working well.

No confirmation of arrival of BBC gear via DHL to Tenerife (see Appendix II). Phil Taylor (RVS Technical Liaison Officer/TLO) indicates that he may also need winch gear brought out from the UK. As a result decide to do multiple RMT deployments in this area until these two issues resolved.

Given motor overheat problems TLO asks for winch to be stood down for a while after RMT hauled. As a result decision made to switch to a lander deployments, with retrieval at 0700 20th Oct. 1999. Master to fax description of activities through to Spanish navigation authorities for continued work in this area. After lander deployed hope to do RMT, if winch allows, with neuston nets and ring nets deployed on davit by scientific team tonight.

1215 Commence hauling RMT; 1300 RMT nearing surface, Closing Cod End (CCE) not closed; 1324 RMT on deck. Catch taken to 10' container on after deck and live shrimp removed under dim red light. Rest of catch then taken to gimbal table for sorting. Many *Cyclothone*, a few small *Chauliodus*, *Gonostoma elongatum*,

*Diplospinus* as well as a few myctophids (*Lampanyctus, Lampadena* and others). Three live *Oplophorus* collected for electrophysiology and placed in cold room. One small *Histioteuthis*, and one *Abraliopsis*. One *Phronima*. Verdict: too shallow (636-788 m) and catch condition not helped by CCE failure.

1603 **Station no. 13632** ISIT lander deployed. Some problems with detaching float on first attempt to deploy, delaying deployment by 30 minutes. Lander followed to sea bed at 40m/min sink rate.
RVS technicians then request to take winch to pieces and hold off further trawling. This agreed, and second RMT cancelled. At 1930 winch ready for test but RVS Team uncertain whether it will work properly. Given time of day, uncertainty over winch, and the fact that the RVS Team have been working solidly on the winch since 0730 (and would be required as winch drivers for the deployment to ca. 2300), PSO cancels RMT work for the day.

Ship then bimbles until darkness when Oxfam net deployed by scientific party. Many *Glaucus*, myctophids and *Halobates*. These filmed by BBC for *Open Oceans* programme of *The Blue Planet* series.

**Wednesday October 20th 1999**

0500 ISIT lander recalled acoustically. On surface at 0635. Pickup on surface without incident, although requiring two passes for grappling. Lander on deck at 0735 and secured by 0743.

Information from RVS re the arrival of the BBC gear to Tenerife not useful. At PSO's request, Master faxed RVS-OPS to reinforce need for active tracing of the package. Feedback by 'phone is that DHL have the BBC gear held in Madrid.

0838 RMT 1+8 CCE deployed to target fishing depth of 1000m. **Station No. 13633.** Water depth *ca*. 3700m.

Seametrix winch electronics still not giving veer/haul or wire out information. RVS Team are anxious about ability of winch to haul: only one motor is working normally, the other taking three times its normal working current and hence over heating. However, the trawl is agreed and they propose to monitor the winch hydraulic pressures during deployment, and then swap a hydraulic pump from the 10 tonne CTD winch to the 20 tonne trawl winch. Before doing so they hope to have confirmation from RVS (Paul Mason, recently returned from *RRS Charles Darwin* and RVS Scientific and Engineering Group's main winch expert) and Lawtons engineering company responsible for the *RRS Discovery* winch system, that their plan is the most appropriate. Pump transfer anticipated to take 5 hours.

0915- 1230 Professor Jim Bowmaker volunteered to invigilate Dave Stewart (*Discovery* ETO) for his Open University examination in pure mathematics, thereby relieving the Master for other duties.
0915-1000 Oxfam ring net deployed by scientific party. Very little in catch.

Winch gave problems during deployment of RMT. Winch stopped when net at 1000m. When net opened (successfully) winch would not veer so net could not be kept at target depth. Slow haul commenced immediately in effort to rectify winch problems and kept hauling because of anxiety of being able to restart the winch. Haul continued so that 2 hour tow planned for 1000m was truncated to 1 hour, and target depth of 1000 m not made (trawl fished between 1000 m and 650 m depth). Winch cable-out monitor now working. CCE then closed (successfully) and RMT brought to surface and in board at 1142. Catch sorted under red light in container on after deck removing live *Oplophorus*, *Sergestes, Gennadas* and *Acanthephyra* as well as many small *Eucopia* Catch then transferred to deck lab and distributed. Catch includes *Eurypharynx* (very small), myctophids, *Chauliodus, Gonostoma, Lampanyctus, Cyclothone, Eustomias, Stomias brevibarbatus* etc.

Fishing then abandoned pending further winch tests, although lander deployment planned for later in the afternoon.

1313 Winch tests with deployment of weight on end of cable. Overheat in winch room led to close down of winch. Test then continued.

1400 Meeting with RVS Technicians, Master, Peter Herring, C/O, & C/E to resolve problems. Decision made to work on winch tonight with aim of producing cure or prognosis for 0600 tomorrow. Meanwhile steam towards Tenerife with the intention of collecting BBC gear and/or engineers from the UK, deploying lander, and conducting winch trials, en route.

1930 Ready to deploy ISIT lander.

1945 ISIT lander deployed. Station number **13634**.

Further winch tests until later when decision made that winch would not be working by 0600 tomorrow and that further tests would be required with advice from Lawtons and RVS SEG. In consequence decided to stay at lander deployment site.

Information received from RVS-OPS that we have received verbal clearance to work in Senegalese waters and that written clearance will follow.

**Thursday 21st October**

0749 Ship heaves to for ISIT recovery, and lander recalled acoustically; 0918 Lander on surface and sighted; 0948 Lander in board after only one attempt to grapple; 1000 Lander secured.

Examination of video tape shows flashes on descent, but no flashes or light apparent on bottom in five 10 minute periods of recording.

The ship then made way to a point at 27º 43′ N 16º 58′ W, just greater than 20 miles from Tenerife island, for an AUDOS lander deployment at station no. 13635, arriving on station at 1500.

Work on winch continues. Trawling not possible.

1855 AUDOS lander deployed and released. Station no. **13635**.

Ship then remained head-to-swell to allow electrophysiology experiments to be conducted on the few shrimps caught in the test RMTs. Experiments continue to 0300 22nd Oct. running concurrently with a party to celebrate Camila Henriques' 22nd birthday.

**Friday 22nd October**

0600 AUDOS triggered for assent; 0705 AUDOS surfaced; 0756 AUDOS on deck; 0806 AUDOS secured.

Ship then made way in flat calm and sunny weather for Santa Cruz. The Lawtons engineer having been delayed by airport chaos in the UK, the planned boat transfer is also delayed. Nevertheless, the launch is alongside at 1748 and, after a somewhat hairy transfer of people and belongings, the engineer and BBC baggage are both on board. David Hunt, who has helpfully volunteered to go ashore to balance the ship's numbers is waved off. The ship then proceeds to the winch test station 20 miles NE of Santa Cruz.

1930 We are beginning to encounter the tail-end of storms further north, with a long heavy swell. Ship maintains head to swell or runs with the swell as possible to minimise roll.

2051- 2230 Two Oxfam net deployments made. *Glaucus*, amphipods and small fish. The only scientific sample taken is for David Hunt, who is ashore.

**23rd October 1999**

Overcast and experiencing a long swell from the north. The engineer, with RVS SEG, help is tackling the winch problem but at 1100, after three hours work including a winch test deployment of some iron shackles, reports that there is "no quick fix" in prospect.

1140 PSO requests lander deployment but this rejected by Master as too many deep-sea cables, and need to keep ship stationary and head-to-swell while winch is being dismantled.

1400 Oxfam ring net deployed from starboard davit. Very little in catch (not even *Glaucus*)

1800 RVS TLO suggests a deployment of the RMT 1+8 CCE to test the winch under operating conditions. RMT readied while work continues on which system.

1918 RMT 1+8 CCE deployed. Station No. **13636**. Winch veered to 1000 m.w.o and net opened. Whilst held winch motor no.2 trips out due to overheating. Trawl aborted prematurely without hitting target depth. Net and CCE closed using hull acoustic transducer. Net hauled with motor no.1 and on deck by 2151. Whilst the meagre catch (*Cyclothone, Chauliodus, Periphylla, Atolla,* few *Sergia* and *Gennadas*) is processed, the RVS technical team stand down for the night.

**24th October 1999**

Weather abated and swell substantially diminished. The ship remains head to swell at 28º 35′ N 15º 36′ W, whilst the RVS team dismantle valve 19 within the winch system, hoping to find and cure a problem with this valve. Work continues all day, with various valves being dismantled and replaced. No fishing possible.

PSO has meeting with Master and TLO to review progress. Situation at 1600 is that TLO has only limited confidence in the winch system, despite the identification of various problems and the rectification of some of them. It is agreed that OTSB work is probably not possible, but that the lander work can continue, and RMTs may be possible. An RMT deployment is planned for later as a test of the winch to greater depths (1000m agreed). PSO proposes that we then organise a "soak test" of the winches, running them for at least 12 hours under normal operating conditions. If they pass this, and if the RVS technical team can give reassurance that the winches are likely to continue to work to this level, with little risk to deployed gear, then we should proceed south. Otherwise the cruise will be reviewed with its termination an option: without a fully operational winch it is clear that the main objectives of the cruise, and the NERC grant with which it is associated, cannot be met. At this point we have lost 6 days of the 26 days of science time allocated to the cruise due to the winch problems (see Section 8).

1820 Station **13637** RMT 1+8 CCE deployed and, with PES outboard, trawl winch veered to 1000m depth. Net opened and veer terminated by quick haul, which, for some unknown reason, decreases current to Motor 2. CCE operated and trawl recovered successfully at 2136 after the planned fishing time. Catch very limited in these oligotrophic waters, but at least we have something to process. Catch includes *Cyclothone, Eurypharynx*, many small *Eucopia, Acanthephyra, Sergia, Gennadas, Meningodora, Atolla* and three very large *Gnathophausia*. Vessel then head to swell to allow work to continue, until 0100 when change of course necessitated checks in all labs to ensure all secure.

**25th October 1999**

Vessel kept head to swell for much of the night, turning beam on at 0850 for the run back to Tenerife. Now confirmed that the pickup will happen at 1200, after which the soak test will proceed, starting at 2400 to allow RVS technical staff a rest period before arrival at the designated site work site to the SW of Tenerife. This site has been chosen to allow room for continuous trawling runs with the vessel being head to swell for a period after the trawls (assuming swell from NW as at present).

1200 Boat transfer off Santa Cruz accomplished without incident. Good to see David Hunt back on board. Vessel then made way to trawl site.

1700 Fax received to let us know that we have permission to work in Senegalese waters, but only if we take on board an observer. PSO determines there is no time for a run into Dakar and thus we will stay out of Senegalese waters unless the observer requirement is withdrawn. Since the OTSB work is the primary interest in these waters, and this trawling is in some doubt, this aspect of the cruise plan will be abandoned. Edie Widder, though particularly fond of Dakar and keen to renew acquaintances, will have to remain at sea…

1200 arrive at trawl site SE of Heirro. Sea state and increased wind make deployment of trawl too risky with CCE and, having assessed situation after ship comes head-to-wind, decide to trawl with open cod end.

**Tuesday 26th October**

Three RMT 1+8 deployments accomplished satisfactorily with some useful animals for processing despite lack of CCE.

0037 Station **13638#1** RMT 1+8. Numerous decapods, and mysids (*Gnathophausia, Eucopia*). Three *Melanocetus, Idiacanthus, Cyclothone, Malacosteus, Lampanyctus* and large *Chauliodus, Eurypharynx* and *Sternoptyx*

0452 Station **13638#2** RMT 1+8. Two large *Eurypharynx*, large *Serrivomer*, scopelarchids and melamphaeid. *Certoscopelus* and many *Cyclothone.* Several *Beroe,* one *Periphylla* and small *Atolla. Eucopia* and two *Gigantocypris.*

0942 Station **13638#3** RMT 1+8. Large *Anoplogaster, Histioteuthis*. Good catch of decapods.

By continuing on head-to-swell course to the NW for all trawling, catch processing continues till 1520 when PSO advises ship to change course to the southern work site at 17º 40′ N 20º 0′ W. Ship rolls on new course.

1530 Meeting with Master, PSO and TLO to discuss winch operation and limitations. TLO reports that it is now possible to control the winch motor currents by doing a quick haul after a veer-stop transition. This is fine at depth, but not when trawl brought on deck and for this reason a manual override to a particular solenoid valve is planned. PSO seeks assurance from TLO that winch will be able to work 24hrs/day with the modifications done. TLO proposes that we start with RMT 1+8 CCE trawls to <1500m depth, followed by increased load for 48 hours. PSO suggests order is RMT1+8, RMT25, OTSB, the latter having more loading on winch and requiring more wire out. TLO quietly confident that even OTSB trawls will now be possible.

ETA at Mauritanian border ca. 0800 Thursday 28th October; ETA at previously identified main work site 2100 Thursday 28th October.

**Wednesday 27th October**

Passage south in fair, sunny weather. Sea state markedly more benign. Following wind revises estimate of ETA at work site to 1700 tomorrow. Plans made for both landers to be deployed, followed by RMT 1+8 CCE hauls throughout the night and the following days, interrupted only by returning to the lander deployment site for recovery in the early mornings.

At midday, we receive confirmation from Senegal that we can work in their waters without an Observer on board.

1512 heave to and then make way at 3 knots for winch test, dropping weighted wire to 450 m to test manually overridden solenoid. Result is surge in current no longer experienced on veer-stop transition and no short haul required after veering.

2045 21º 57.4′ N 18º 37.1′ W Oxfam ring net deployed in area of high bioluminescence. *Pleuramama* common, along with small hydro-medusae.

**Thursday 28th October**

Passage south in calm, hot and very sunny weather. Many flying fish, both two-winged and four-winged species. Gentle swell and wind from the north still assisting our passage and 14.5 knots over the ground until 1200 when reduced speed to 10.5 knots due to engine overheat. The sea temperature is now 25-28ºC and both the engine cooling and A/C is struggling. ETA at the work site fluctuates from 2100 to 1600 to 2000 as the day progresses.

Arriving at 17º 40′ N 20º 00′ W at 2024 both AUDOS and ISIT landers are deployed in rapid succession (Stations **13639** and **13640**). By 2200 the first RMT 1+8 CCE (**Station 13641#1**) is veering and through the night two trawls are made, providing useful catches for all. The first net, delayed by main winch at first refusing to veer until hauled by deck winches, returns several large jellies, *Atolla, Periphylla, Beroe*, and many *Eucopia*, with a few *Meningodora* as well as large *Notostomus* and

*Acanthephyra* but few *Oplophorus* or *Sergestes*. Large *Malacosteus, Borostomias, Lampadena*. One *Sternoptyx*. The second net (**Station 13641#2**) includes large salps (*Thetis*) and four spectacular *Stylephorus* as well as a small tube-eyed *Gigantura*. Some large *Diaphus* and several large *Lampanyctus*. Also *Chauliodus*, *Argyropelecus*, one *Malacosteus*, *Serrivomer* and *Cyclothone*. A few *Acanthephyra*, *Sergia*, *Gennadas*, and large *Mastigoteuthis* and small *Histioteuthis*. One small but very lively *Melanocetus johnsoni* provides useful footage for the BBC before its esca is committed to molecular biology.

The winch is working reasonably well and electric currents to the motors are within acceptable bounds.

At 0700 the ship is back at the lander site and the ISIT lander is recalled. Surfacing at 0820 it is on deck and secured by 0854. Although the 'splat' screen is slightly displaced, the tape reveals bioluminescent flashes on the sea floor at 3231 m depth. At last, deep benthic fishes do have light to look at…

**29th October 1999**

Trawling throughout the night in excellent conditions yielded useful specimens (above). Daytime trawling continued in very hot conditions with equally useful catches. The first (Station no. **13641#3**) included *Argyropelecus gigas*, a few *Vinciguerria, Diaphus, Serrivomer, Sternoptyx* and a tiny *Malacosteus*. Crustaceans included *Systellaspis, Sergia, Sergestes* and some *Gennadas*. The last trawl (**13641#4**), on deck in late afternoon, featured with a very large (> 1 m long) lancetfish (*Alepisaurus ferox*) which was caught in the CCE ball valves. Although an interesting fish, usually only caught by long-lining, and quickly demolished by scientists and the galley, it blocked the flow of water into the net and most of the catch, apart from some hardy *Gigantocypris*, was d.o.a.

Trawling was then interrupted for an ISIT lander deployment (Station no. **13642**) before recommencing. An evening trawl (**Station no. 13643#1**, to 200-320 m) proved excellent for *Gonostoma* and shrimps (*Systellaspis debilis, Acanthephyra*) but a large *Pyrosoma* blocked the CCE and most of the shrimps were dead.

**30th October 1999**

A deep tow targeting 1500m (**13643#2**) in the early hours yielded little, and included a lot of wash down from the previous trawl, but contained three small angler fish as well as a large *Galiteuthis* and a small *Histioteuthis.* Also *Cyclothone, Parabrotula and Leucobrotula.*

The ship then returned to the lander site and the AUDOS was released at 0605, was on the surface at 0712, and on deck at 0747. The ISIT, which was released when the AUDOS was on the surface was on the surface at 0910 and on deck at 0945.

The long swell has reduced but is now replaced by increased waves, wind 6-7, and occasional rain under an overcasts sky. Nevertheless, trawling with the CCE was still possible and a midday trawl to a targeted 750m (**13643#3**) was excellent for fish, including four live *Malacosteus*, readily video-taped and later used for a multitude of purposes.

In the early evening the net was pulled to one side of the afterdeck to allow deployment of the AUDOS lander (**Station no. 13644**) and at 1910, before the night watch came on duty, the RMT 1+8 CCE was back in the water (**Station no. 13645#1**) and fishing between 400 and 500 m depth. This proved a useful depth at this time of day, and many interesting fish were caught, including *Gigantura, Stylephorus, Argyropelecus* spp., *Gonostoma, Cyclothone* and one male angler fish. Among the crustaceans *Acanthephyra* dominates, with *Sergia* and *Gennadas*.

A short trawl in the late evening (**Station no. 13645#2**) to 200-300 m was intended to provide live shrimps and did so, especially Sergestes, Sergia and Gennadas. Fish included *Melanonus, Argyropelecus, Gonostoma, Diaphus* spp. and *Valenciennellus*.

In the middle of the night a somewhat deeper trawl (**Station no. 13645#3**) to 500-600 m proved excellent for angler fish, providing three species, *Chaenophryne, Oneirodes,* and *Melanocetus*, in addition to live *Stomias*, a few myctophids, *Serrivomer, Cyclothone*, one *Howella* (the first caught), *Scopelarchus analis* and *Anoplogaster cornuta*.

Towards the end of the day a plan for the rest of the cruise was drawn out , to allow RMT, lander, and otter trawling in equal measure at three specific sites. These sites span the 3250m deep plain currently being worked over, a 4000 m deep water site to the south, and a shallower site, ca. 2500 m deep, on the African continental slope (constituting the 'Mid', 'Deep' and 'Slope' sites respectively). Such plans are, admittedly, only ambitions at this stage but the positions have been chosen to maximise the diversity for lander and OTSB deployments. The "Mid" site, at 17º 40′ N 20º 00′ W is approximately equidistant (150 miles) from the "Deep" site, at 15º 00′ N 20º 30′ W and the "Slope" site, at 18º 45′ N 17º 50′ W but not on a direct path between the latter. A mid point on the steaming path between deep and slope sites is identified (16º 53′ N 19º 10′ W) but the detour back to the mid site is only 40 miles (*ca*. 4 hours steaming) and, in the interests of better sampling design, we will return here for OTSB work if the latter proves possible.

**31st October 1999**

Recovery of the AUDOS lander in the early morning emphasised the state of the weather, now wind force 8 with a high sea and unpredictable swell. Fishing with the CCE was quickly abandoned and a decision made soon afterwards to spend the day in passage to the identified 'Deep' station at 15º 00′ N 20º 30′ W. Although there is insufficient weather information to make a fully rational decision that we will face better fishing prospects further south, it is preferable to sitting idle at this latitude. At present the weather is border-line for lander work, impossible for use of the CCE, and less than ideal for general fishing particularly as veering a light load in a heavy swell is potentially dangerous. By 0940, after a lab inspection by the PSO and others to ensure that all vulnerable gear was properly stowed, the ship was steaming south.

As the day progressed, so the weather improved, with occasional bursts of sun shine in an otherwise overcast sky, and lessening in sea.

**1st November 1999**

A cruising speed of 9 knots to the deep water work station at 15º 40′ N, 20º 30′W had the ship in position at 0412 for a rapid bathymetric survey to check that the sea floor was suitable for OTSB deployment if that proves possible; principally an absence of canyons or sea mounts. Weather conditions now excellent for work: sea more or less flat and an absence of wind. At 0724 the AUDOS lander was deployed (**Station No. 13646**) for its excursion closest to the equator (15º N). Afterwards the RMT 25 is readied for action. This net has no acoustic monitor or control, nor CCE, and is fished open. At 0854 this was shot (**Station No. 13647#1**) and by 0900, with 1900m of wire out the winch veer was ended and the trawl continued for 1.5 hours.

Hauled at 15m/min,rather than 30m/min used for the RMT 1+8, to minimise catch damage, it was disappointing to recover the net with most of the catch caught up in the net, and very little in the cod end. Clearly the net either tangled, or the lazy line (used to recover the cod end without fully recovering the net) was looped round the body of the net. As a result the catch was in very poor condition but the provision of large amounts of sample satisfied many on board. The catch included huge quantities of *Pyrosoma*, many meters long, which will have seriously impeded the catch flow into the cod end even without the twist in the net, pelagic ascidians, 20 *Chauliodus*, four *Malacosteus*, *Scopelarchus analis*, *Melanocetus, Melanonus, Gonostoma*, also, among the cephalopods, *Vampyroteuthis, Vitreledonella* and *Histioteuthis*. It is difficult to tell how promising this depth and location will prove to be from this trawl, but the winch worked well and electric currents to the motors remained within sensible bounds on veer, stop and haul.

After an attempt to flush the net by deployment without the cod end in place the net was re-deployed (**Station No. 13647#2)** soon after 1400. During the trawl an

Emergency Drill was conducted but this did not interrupt fishing and close to 2000 the net was back on deck, this time with a exceptionally huge and excellent catch which more than satiated even the most voracious molecular biologists and biochemists. Many *Sternoptyx, Argyropelecus, Chauliodus and Gonostoma* led to a production line of processing for pineal anatomy and mRNA studies. Also, and various melanostomiids such as *Flagellostomias, Borostomias*, as well as myctophids and gonostomatids. Highlights of the catch included numerous *Malacosteus*, as well as *Pachystomias, Aristostomias, Dolichopteryx, Stylephorus, Saccopharynx, Eustomias* among the fishes. A large variety of cephalopods were also caught, some of which were filmed by the BBC, but also *Vampyroteuthis, Chiroteuthis, Mastigoteuthis*. Among the crustaceans both *Notostomus* and *Acanthephyra* were abundant, but also *Ephyrina*.

The large catch took a long time to sort and process but the net was re-deployed to *ca*. 300 m depth (**Station no. 13647#3**) at 2130. After an hour at depth the incoming catch was relatively disappointing in terms of volume but notably produced *Chiroteuthis, Vampyroteuthis, Japatella, Vitreledonella*, and many live larval cranchid squid. Also *Atolla* and *Periphylla* in excellent (live) condition, as well as ctenophores and medusae, and large salps as well as the usual large mass of Pyrosoma. Main species of shrimps *Notostomus*.

The ship is alive with insects, especially green bugs (which do not conform to familiar vegetarian habits and bite savagely), which are presumably in transit from the Cape Verdes to or from mainland Africa.

**2nd November 1999**

An early morning recovery of the AUDOS lander in flat calm conditions was efficiently conducted and quickly followed by shooting the RMT 25. This haul, (**Station no. 13647#4),** after an hour fishing at 1200 m.w.o. (ca. 600 m depth), yielded many *Chauliodus, Photostomias, Sternoptyx, melamphaids, Borostomias, Photostylus*, and *Dolichopteryx*. Small cranchids, *Sergia, Notostomus, Acanthephyra, Mastigoteuthis, Vitreledonella* and masses of large soft *Pyrosoma* and large salps (*Thetis*?) The fish are in excellent condition as they have been in all but the first RMT 25 trawls, possibly protected from abrasion by the gelatinous pyrosomes, or perhaps it is due to the excellent fishing conditions: flat calm and sunshine.

The second RMT 25 deployment (**Station no. 13647#5**), which took most daylight hours, was sent deep both in an attempt to catch bathypelagic species and also as a test of the winch. Paying out 4000 meters of wire, to an estimated fishing depth of 2000 m the trawl was slowly hauled for a period, and then fished at this depth for one hour. The catch was recovered after a total of eight hours trawling at ca. 2000 m depth and was in excellent condition with remarkably few salps and no *Pyrosoma*, putting pay to the idea that gelatinous animals protect the catch. Fish included *Opisthoproctus* in good condition*, Malacosteus* (live), *Aristronesthes, Dolichopteryx, Melanonus* and various melamphaids and tiny searsids. Crustaceans included *Hymenodora, Sergia, Acanthephyra, Systellaspis*, two species of *Gigantocypris*, and others, whilst the cephalopod list included *Vitreledonella* and *Grimalditeuthis* as well as *Vampyroteuthis* in good condition. Not only an interesting, if not voluminous, catch, but also the winch performed well with the greater weight of wire out.

After the trawl the net was craned out of the way for a deployment of the ISIT lander (**Station no. 13648**) which was rigged as the ship steamed back to the lander location. Once away, the ship steamed down-swell from the lander site for half an hour while the RMT 1+8 CCE was rigged to provide live animals for filming and for on board physiology. This net (**Station no. 13649#1**) was shot at 1926 and opened at 624 m depth, which in previous deployments had proved good for both fish and crustaceans. Despite this, the trawl yielded no cephalopods and only a relatively poor catch of mostly standard species of fish (*Chauliodus etc.),* although these included some live melamphaids and a live *Melanonus zugmayeri.* Nevertheless, those interested in dark-caught shrimp benefited from *Notostomus*, many *Acanthephyra*

and a few *S. debilis*. During the trawl a problem with the winch (the Cobra main traction unit tripped out) prevented veering, but power was soon re-instated and the winch was veered and hauled to maintain a steady fishing depth. The Cobra failure was apparently a feature of cruise D242, so let us hope that this does not continue.

After re-rigging the net was re-deployed (**Station no. 13649#2**) within half and hour by the night watch and sent to 400-500 meters in order to target more shrimp. On recovery this proved to be a mistake, the catch being very poor, with only two *Acanthephyra*, and dominated by a single large octopus and mangled siphonophores, with few vertebrates or crustaceans.

**Wednesday 3rd November 1999**

The next RMT 1+8 CCE (**Station no. 13649#3)** was shot at 0130 and veered to just over 350 m.w.o to fish close to the surface, a depth rarely fished on these cruises. The catch was surprising, containing hatchets, gonostomatids, and several *Astronesthes*, but the curiosity of the catch was undoubtedly a large "4-eyed" opisthoproctid fish *Bathylychnops*. Shrimps included *Gennadas* and *Systellaspis* with some euphausids and a few *Sergestes* After the net was cleared the vessel steamed for two hours back to the lander site and at 0540 the ISIT lander was released, surfacing at 0714 and being in board at 0749. The current meter was then swapped from the ISIT to the AUDOS lander and the latter was deployed (**Station no. 13650**) at 0921. The vessel then ran south while the RMT 25 was prepared and this was launched (**Station no. 13651#1**), after a short winch delay, just after 1000. After fishing around 1200 m depth the trawl was in board by mid afternoon, yielding a spectacularly catch including several live *Chauliodus*, three *Malacosteus, Pachystomias, Borostomias*, and *Platytroctes*, as well as various myctophids, melamphaids, *Nemichthys* and *Sternoptyx*. Crustaceans were unusually diverse, including *Gigantocypris, Systellaspis debilis*, and *S. cristata, Acanthephyra, Meningodora, Sergia, Eucopia, Eurythenes* and pasiphaeids.

The next net, (**13651#2**) was designed to sample very shallow water, to around 200 m depth, to see if euphausids are present in any significant numbers in the day time. If so, CCE trawling will be conducted tomorrow. In the event the net returned only a small catch of familiar animals. It seems that 200m is not particularly 'shallow' in this region, presumably because the light attenuation is so high. The last trawl of the day (**13651#3**), shot in late afternoon to 700 m was, again, spectacular in quantity and diversity, the fish including *Malacosteus, Pachystomias, Winteria, Anoplogaster* and three ceriatoid angler fish as well as the more common stomiids and hatchet fish. Shrimps similarly included the common species, but also *Notostomus* and *Eucopia*. Cephalopods were also diverse: *Japatella, Bathyteuthis*, several small and live cranchids, several *Vampyroteuthis* (skinned), *Mastigoteuthis* and *Ornithoteuthis*.

Fishing then switched to the RMT 1+8 CCE for night work, the first deployment (**13652#1**) aiming for euphausids at 500-700 m. An undefined problem with the net prevented a full catch and much of the contents of the cod end were wash-down from previous trawls. Nevertheless, two large ceriatoid angler fish were recovered in perfect condition, as well as *Malacosteus, Pachystomias* and *Scopelarchus* (with another in the RMT 1).

**Thursday 4th November**

After repairs to the net the next trawl (**13652#2**) was deployed at 0200 and was hauled during the trawl to fish between 300 m and the surface. This caught mainly small fish, including tiny *Stomias*, *Chauliodus* and hatchetfish, as well as a few crustaceans including many euphausids, *Plesionika* and *Sergia*.

After the net recovery, preparations were made for the lander recovery, the ship steaming to the station for a period and as dawn broke for another day of perfect conditions for lander work and trawling: long low swell and wind force 2-3, the AUDOS was released at 0600. On the surface at 0739 it was in board at 0812. The ship then steamed south of the lander position until 0900 at which time the RMT 1+8 was ready to shoot. A slight hold up when the cod-end lift line was lost was quickly overcome with some expert grapple throwing (now well practised with the lander recoveries) and the net (**Station no. 13652#3**) was shot at 1010 to fish close to 1000 m. On retrieval the catch proved to be very light, with no live shrimps at all (but several species), a school of tiny melamphaids, and one spectacular, large and live *Melanocetus* angler fish *with* an attached male. This rare find was avidly video-taped by the BBC and others before being preserved for a museum collection. The lack of live crustaceans indicated a problem with the CCE ball valves and, on investigation, it was found that the aft ball valve was not held properly closed because it was turning beyond its stop.

The next station (**No. 13653**) was for a "Bungee ISIT" deployment in which the lander was deployed with a downward facing video camera, imaging a netting "splat-screen", reached the sea floor and was immediately released for recovery. The resultant video showed bioluminescence all the way down through the bathypelagic but, unfortunately no video was obtained as the lander hit the sea floor.

Immediately after recovery of the ISIT, the AUDOS lander was prepared and deployed (**Station no. 13654**) before attention returned to trawling with the RMT 1+8 CCE. The first haul (**Station no. 13655#1**) to 800-1000 m was dominated by jellies, mainly siphonophore with *Periphylla* and *Beroe*. Fish included *Scopelogadus, Stomias, Photostylus* and many *Cyclothone*, but no other notables. Crustaceans included the familiar gamut (*Gigantocypris, Sergia, Acanthephyra* and many *Eucopia*) but cephalopods were restricted to one large gelatinous octopod.

**Station 13655#2** was recovered just after midnight and was an extremely shallow trawl (52-300 m depth). Interestingly it caught many small but familiar deep-water species including *Stomias, Chauliodus, Vinciguerria, Sternoptyx*, melamphaids as well as two small ceriatoid angler fish and several leptocephalus larvae, as well as familiar crustaceans.

**Friday 5th November**

The next trawl (**13655#3**) fished deeper (530-700 m) throughout the early hours of the morning and included some very interesting animals (two *Malacosteus, Opisthoproctus* in particular, and *Astronesthes* in perfect condition) as well as the familiar fish. *Acanthephyra, Notostomus* and *Meningodora* dominated the crustaceans.

Trawl **13655#4** was deeper still, close to 1200 meters, and was notable mainly for problems with the winch. Deployment was delayed as the winch system shut down, the warp left the cable haulers (luckily with no damage to the wire), the Cobra traction system then shut down spontaneously once again (as it had done the previous day), and finally winding motor 2 overheated and tripped out during recovery. Fortunately the trawl was recovered and was notable for a large *Saccopharynx* in perfect condition, with full gut. This was carefully filmed by the BBC and then fixed for later work.

Because of the problems during this trawl it was decided to work on the winch during the day. To minimise idle time the ship returned to the lander site, the AUDOS lander recovered, and the ISIT prepared for deployment. While the RVS technicians once again laboured in the winch room and kept the 'phone hot to Southampton and Lawsons, a pod of pilot whales visited the ship providing some light relief for the anxious scientific party.

Following a successful winch test involving veering a short length of trawl warp onto the after deck, assisted by one of the deck winches, the ISIT lander was deployed (**Station no. 13656**). Thus, despite the down-time due to the winch, the impact on science time was minimised and the loss of scientific work kept to 3 hours.

Consultation with the RVS technicians suggested that an RMT25 deployment would be the most appropriate test of the winch. This net was deployed in late afternoon (**Station no. 13657#1**) and recovery close to midnight after a trawl at an estimated 1000 m yielded an excellent and diverse catch, although somewhat dominated by pyrosomes and salps. In addition to many *Chauliodus, Malacosteus, Stylephorus, Scopelogadus, Photostylus, Stomias*, hatchets and *Chiasmodon* (with grossly distended stomach) the catch also included *Eurypharynx, Aristostomias*, a male ceriatoid angler fish, and a perfect female angler which lived for a considerable time and showed repeated "casting" of its ilicium/esca. Crustaceans included many common decapods, *Sergia, Acanthephyra, Notostomus*, as well as numerous *Gigantocypris* and *Eucopia*. Another gelatinous octopod was accompanied by many small cranchids, *Mastigoteuthis* and *Bathyteuthis*. Fortunately, the winch worked perfectly.

A second deployment of the net (**Station no. 13657#2**), to a slightly lesser depth (*ca*. 700 m), was equally successful, and included *Borostomias*, hatchets, *Chauliodus*, searsids, *Stomias, Melanonus, Photostomias*, a live *Nemichthys*, melamphaids, *Malacosteus*, and leptocephalus larvae. Star of the fish catch, however, was a large *Bathylychnops* which was carefully preserved. Crustaceans included *Acanthephyra, Sergia, Eucopia, Sergestes* and others, but, unlike the fish, these were in relatively poor shape. Once again the winch worked well.

**Saturday 6th November**

Following retrieval of the net the ship steamed to the lander site and the ISIT was recalled at 0528. Arriving on the surface at 0653 it was deemed prudent to hold off retrieval while the light improved, but nevertheless the lander was in board by 0741. The retrieval was once again helped by a flat calm sea and wind force two or less. The weather further north is also improving and so a move to the 'Mid' or 'Shallow' sites is now possible. However, before deciding it would be preferable to reassess the possibility of an otter trawl deployment at this site and a full day of winch work is needed to re-evaluate the reliability of the winch. If the OTSB is possible, it will be necessary to recover it in daylight in case of problems.

At 0836 the lander was cleared from the aft deck and RMT 25 (**Station no. 13657#3**) was shot to 1200 m depth. This net was deployed at the standard 30m/min veer rate with the ship moving at 2kts, thus fishing at 1kt through the water. When at depth, however, the standard 2kts was reduced to 1.5kts and hauling proceeded at 10m/min instead of the usual 15m/min, again keeping the ship's speed to 1.5 kts instead of 2kts. The intention of this procedure was to see if gentle trawling noticeably improved the quality of the catch. On recovery in mid afternoon the catch was extremely heavy and in excellent condition, and included a very large *Melanocetus, Pachystomias, Scopelogadus, Nemichthys, Serrivomer, Stomias, Bajacalifornia* and other alepocephalids and searsids, *Platytroctes, Malacosteus* (which seems to appear in most nets!), *Sternoptyx, Opisthoproctus, Photostomias* and a live *Anoplogaster*. Crustaceans included *Gnathophausia, Gennadas, Sergestes, Acanthephyra, Eucopia, Sergia* and others. Cephalopods included *Mastigoteuthis, Japatella* and a large transparent and gelatinous octopod. Once again many salps, medusae, siphonophores and pyrosomes accompanied the catch. The consensus of the scientific party was that this method of trawling produced an unquantified but general improvement in catch condition. Apparently the slow trawling does not reduce the amount of catch of commonly caught species, but a noticeable improvement in condition was apparent. If we had a closing cod end on this net we would have a truly excellent system.

After the net recovery a short steam to the lander deployment site was followed by a straightforward deployment of the ISIT lander (**Station no. 13658)** and

was followed by a switch to the RMT 1+8 CCE trawl, a series of deployments being planned to target euphausids for electrophysiology.

The first, (**Station no. 13659#1**) to *ca*. 700-800 m produced a very large *Stomias*, many *Sternoptyx, Melanonus, Hymenocephalus* and small *Synaphobranchus*, and *Chauliodus*, as well as *Cyclothone*. Once again a large gelatinous octopod was caught, as was *Japatella*. Crustaceans included many live *Gigantocypris , Eucopia, Sergia, Systellaspis* and *Acanthephyra*.

The next net (**Station no. 13659#2**), slightly shallower (300-603 m) and deployed in darkness produced similar fish but crustaceans were dominated by *Sergia* and *Acanthephyra*, with some *Gennadas* and *Plesionika* as well as a few euphausids.

In the early hours a third net (**Station no. 13659#3**), shallower still (201-401 m), produced a very large catch of euphausids, our best catch of these yet. In addition, crustaceans included *Plesionika, Sergia, Gennadas, Acanthephyra* and fish notably included *Scopelarchus*, and (for a change) *Gonostoma* in addition to *Sternoptyx, Bonapartia, Chauliodus, Valenciennellus*, and *Cyclothone*.

**Sunday 7th November**

A fourth and final net (**Station no. 13659#4**) in the series rounded off a busy night by fishing extremely shallow (50-300 m) and being recovered just after 0330. Once again small fishes predominated, including *Chauliodus, Sternoptyx, Astronesthes*, small *Aristostomias, Scopelarchus* and myctophids. Many euphausids were caught, as well as *Sergia*, and *Systellaspis debilis*.

A steam to the ISIT station was followed by a recovery in perfect conditions which started the day well. This was quickly followed by an RMT25 trawl to 800m (**Station no. 13660#1**). Once again, slow trawling was shown to improve the condition of the catch and several superb specimens of some unusual species were recovered. These included several angler fish (*Linophryne* and *Oneirodes*) as well as large *Omosudis*, whale fish, and alepocephalids, as well as *Stomias, Serrivomer, Chiasmodon, Platytroctes* and *Scopelarchus*. In all, an unusual collection.

During the first trawl a plan for the next two days was drawn out and, because the winch is working well, this included OTSB deployments. The latter are timed to allow two RMT 25 trawls today, and such that critical times of the OTSB trawl (launch, bottom time and recovery) occur when the most experienced personnel are available. Because the weather to the north is now improved, with low pressure regions moving out into mid Atlantic, a passage to the “Mid” site is planned after the OTSB and subsequent lander recovery.

The second deployment of the RMT (**Station no. 13660#2**) was made speedily after the first and the net was veering three minutes after recovery having brought the cod end i/b and swapped the bucket – surely a record ! The net was restricted to a depth of *ca*. 500 meters so that it could be recovered by 1700, this allowing time for a last ISIT lander deployment at this site, and a the rigging of the OTSB14. This depth proved interesting, once again producing several angler fish (*Linophryne* and *Oneirodes*) as well as *Stomias, Nemichthys, Astronesthes, Scopelarchus, Sternoptyx, Dolichopteryx*, small melamphaids and *Vinciguerria*. Crustaceans included *Gennadas, Sergia, Acanthephyra, Plesionika and one Cystisoma*. Jellies included many *Atolla* and *Beroe* as well as pyrosomes.

A short steam to the lander position was followed by a deployment of the ISIT lander (**Station no. 13661**) in the early evening, again to produce a profile of bioluminescence below 500 meters depth.

Attention then turned to the preparation of the OTSB14 which was rigged by Ben Boorman and Nigel Merrett. By running SE to a deployment site we were also able to check the ground with the PES and ensure that the trawl missed the several canyons that cut down through the continental slope and extend onto the abyssal plain at this latitude. Once on station the ship was turned into wind and the net was deployed (**Station no. 13662#1**) at 2112, the winch veered for four hours, and the net registered the bottom in the early hours of Monday 8th November as planned.

**Monday 8th November**

In the event, the first OTSB trawl of the cruise was not a success though, ironically, the winch worked perfectly. Although care had been taken to deploy the net such that it hit the ground in a suitable place a combination of circumstances (winch veering at an unexpected 50m/min, and the net sinking unusually fast) placed the net on the bottom prematurely. As a result a 100m deep canyon was encountered below the ship and in the path of the net soon after the net hit bottom. Thus a three hour tow had to be reduced to 1 hour in order to get the net off bottom before the canyon. Fortuitous, this was to our advantage as on retrieval just before breakfast it was discovered that the net had failed to fish properly, although it had clearly made bottom. It is likely that this had occurred because of hydrodynamic collapse at the time of deployment, either or both because the ship's speed had been too low or because winch veer had been too high. The catch thus consisted only of a few holothurians, sponges and glass sponges as well as two whale ear bones, and one surface fish. A decision was made to repeat the trawl in the area where the bathymetric survey of (1st November) at the lander site had been conducted (15º 40′ N, 20º 30′W). This we know to be free of canyons and the chance of accidentally catching the lander ballast was deemed to be small. In addition, the surveyed track at 315º although slightly off head to wind/swell would be tolerable and the track is along a contour.

The vessel thus returned to the ISIT recovery position and the lander was brought back on deck in the late afternoon. As it was recovered a turtle investigated the lander floats before being accidentally 'turned turtle' by the lander's mooring rope, at which point, having recovered its dignity, it fled. The ship then headed to the start of the trawl run, some 15 miles SE of the anticipated point at which the trawl will hit the sea floor. Although the swell has increased and the wind is now force 4-5 this is well within the conditions suitable for the OTSB.

At 1347 the OTSB was again shot, reaching bottom in the early evening and being recovered close to 3am after a total trawling distance of 8.2 miles on the sea floor.

**Tuesday 9th November**

The OTSB catch proved excellent, although it had not made it into the cod-end and was caught up in the body of the net. Fish included two very large *Histiobranchus bathybius*, two *Bathysaurus mollis*, *Lionurus, Rinoctes, Conocara salmonea, Bathypterois*, *Chalinura profundicola, Coryphaenoides (N.) armatus*. The trawl was useful in ground truthing the AUDOS photographs but also provided essential tissue from average ocean depths for several projects. Having sorted the invertebrates (which included several *Plesiopenaeus*, many holothurians and asteroids) from the fish the latter were rapidly weighed, measured and shared by the assembled company with only some surplus *C.(N.) armatus* being left for the galley. Work on tissue continued for some until 0930 during which time the ship steamed to the 'Mid' site at 17º 40′ N 20º 00′ W

The wind is now freshening again, to force 4-5, but the swell is low and the prospects for the next few days look good, with a weak high further north. We can thus anticipate the area will be workable for landers and fishing although, according to the Africa Pilot, this weather is far worse than average for this time of year in this area (stated average is wind force 3).

On arrival the AUDOS lander was deployed efficiently (**Station no. 1366**3) at 1915 after which the ship held station while line fishing for lemon-back squid for molecular biology and biochemical studies. Nevertheless, by 2200 the OTSB was deployed (**Station no. 13664#1**) and veering to the sea floor.

**Wednesday 10th November**

Although the electronics indicated that the net had not hit bottom, which caused the night watch some anxiety, in fact it had and, on recovery at the civilised time just after 0800, a good catch was brought on deck. This was notable for the very many hexactinellid glass sponges, like small potatoes, as well as many

asteroids and ophiuroids and holothurians. Several large *Plesopenaeus, Munidopsis* and *Munida* as well as hermit crabs, barnacles, large anemones and solitary corals were also present. Among the fish *Histiobranchus, Chalinura, C. (N.) armatus, Lionurus, Bathysaurus, Rinoctes* and *Halosauropsis* provided, once again, plenty of tissue which was divided and worked on with urgency.

Following the trawl the ship returned to the lander deployment site and at 1230 the AUDOS lander was on the surface and collected safely and efficiently in record time (22 minutes from surfacing), 2nd Office Syd Sykes breaking in the process the previous time record for quick recovery (also held by him).

The weather is still wind force 4-5 wind, but possible abating, and with a low swell. It is thus fine for trawling and lander work and after the first trawl a decision was made to stay at the Mid station and abandon the inshore, 'Slope' station. Given the tightness of the remaining schedule the passage to the slope site is not the most efficient use of ship time. This, and the fact that the wind is likely to be stronger inshore (not to mention that we would be working in an area of fishing lanes and underwater cables) makes work at the Mid site preferable.

Having made this decision, the RMT 25 was deployed **(Station no. 13665#1)** to 1000m depth. As with the previous few RMT 25s the net was towed at 1.5 kts for 1.5 hours and hauled at 10/min. The result was spectacular, with a wonderful catch in excellent condition (although few shrimps survive) remarkably free of pyrosomes. In addition to the usual *Sternoptyx, Chauliodus*, hatchetfish, melamphaids, and *Lampanyctus*, the catch contained some less common *Platytroctes, Borostomias, Bonapartia, Anoplogaster*, and some unusual animals such as *Stylephorus, Saccopharynx, Aristostomias, Pachystomias, Malacosteus, Rhynchohyalus* (juvenile), *Eurypharynx, Melanocetus, Cryptopsaras* and *Opisthoproctus*. For once, *Oplophorus* was in the catch, as were other crustaceans including *Systellaspis debilis* and *S. cristata, Acanthephyra, Sergia, Gennadas, Gigantocypris, Meningodora*, and *Phronima*. Cephalopods were restricted to *Bathyteuthis, Japatella*, various cranchids and one large octopod.

As the catch was sorted the AUDOS was readied and then deployed **(Station No. 13666)** within an hour of the catch being on deck. As the weather is good enough at present for the use of the closing cod end the RMT 1+8 CCE was next readied and trawls planned to target *Chauliodus* for pineal studies and angler fish in an early net, and euphausids in later trawls.

The first net (**Station no. 13667#1**), fished around 600 m, caught a useful catch of *Gonostoma* (rarely caught here), *Cyclothone, Stomias*, a large *Melanocetus* with parasitic copepods, and a large *Winteria*. This depth appears to be a "good" jelly depth at this time and the catch contained several *Atolla, Periphylla, Beroe* and other large ctenophores. This was succeeded by two more deployments which trawled between 300m and the surface and which took a tired night watch into the early hours. Notably the first found *S. debilis* (a target species) and the second brought up various leptocephali, *Melanocetus, Cryptopsaras,* and small hatchetfish, with numerous euphausids (the main target), *S. debilis, Gennadas, Sergestes* and a few *Acanthephyra*. Jellies were also present but not problematic.

**11th November 1999**

The last RMT was on the surface rather earlier than originally planned due to the fast turn around (less than 30 minutes) between nets during the night, and even after a steam to the lander site a two hour idle period was unavoidable. The lander was, however, recalled at 1000 at which time it had had 12 hours on the sea floor. In order to re-synchronise activities so that the lander recoveries can occur at daybreak in future the AUDOS was immediately readied for re-deployment and launched **(Station no. 13668)** some five miles from the previous deployment point. The weather is now perfect for fishing: no swell and wind now force 3-4, which is just enough for the flying fish. Sun extremely hot.

Deploying the RMT 1+8 CCE (**Station no. 13669#1**) with the intention of two nets before evening there was, in the event, only time for one net. The catch was surprisingly small, perhaps attributable to a short (1 hour) tow with no large fish

except one *Scopelogadus*. Crustaceans included *Eucopia, Sergia, Meningodora* and *Acanthephyra.*

The day watch then rigged and deployed the RMT 25 (**Station no. 13670#1**) which was veered to 1600 m.w.o. to fish close to 800 m depth. When attempting to haul, however, a loose turn was noticed on the winch drum. In order to clear this the winch was veered to 2617 m.w.o. before hauling and thus the trawl time was extended by some 3 hours. Surprisingly the trawl, which was recovered at 0318, was *excellent* as well as large, with many live fish despite the large pyrosomes and *Atolla*. Notable were the anglers *Melanocetus, Cryptopsaras,* and *Chaenophryne,* as well as *Pachystomias, Malacosteus,* a large *Gigantura, Stylephorus, Scopelogadus, Gonostoma, Chauliodus, Nemichthys, Serrivomer, Scopelarchus, Ichthyococcus, Photostomias, Platytroctes,* hatchets and a few myctophids. Several species of cephalopods were also present including a large *Vampyroteuthis, Bathothauma, Mastigoteuthis.* Crustaceans included *Notostomus, Hymenodora, Meningodora, Sergia, Eucopia and Gigantocypris.*

**12th November 1999**

Because of the extended trawl it was not possible to deploy another net if the lander was to be recovered in the early hours. Thus, as the haul was sorted, the vessel turned to proceed to the lander site and the AUDOS was pinged for recovery at 0521 and was on the surface at 0645 and in board at 0737. To keep the synchrony of lander recovery the AUDOS was quickly prepared and launched (**Station no. 13671**) at 0930. After a short steam during which the OTSB was made ready, this too deployed (**Station no. 13672)**, and three and a half hours later, at 1335, the net was on the sea floor. After fishing for 3 hours the net was brought off the bottom and was on deck at ca. 2100. The aft deck lights were turned off to allow fish to be removed from the haul in semi-darkness for work on visual pigments, and the rest of the catch was then whisked into the deck lab for sorting and tissue collection. The catch contained many fish and invertebrates, the latter including small sponges, asteroids and a few holothurians. Numerous small *Munida/Munidopsis* as well as a few large specimens. Many large *Plesiopenaeus*, as well as barnacles, hermit crabs, gastropods and a few bivalves and polychaetes. Fish were dominated by *Coryphaenoides (Nematonurus) armatus*, but also represented were *Bathysaurus, Histiobranchus, Bellocia, Bathyonus, Rinoctes* and *Bassozetus*. Prize of the catch was a very large cirrate octopus (*Cirroteuthis*?), approximately 1.5m long, which was much photographed before tissue samples were removed and the whole animal preserved for return to Martin Collins in Aberdeen.

As the scientific “feeding frenzy” proceeded, demolishing the OTSB catch to bony remnants, the RMT 1+8 CCE was prepared and this was shot soon after 2300 (**Station no. 13673#1**) to 400-500 m depth. This apparently missed the migratory animals and the catch was small, being dominated by small medusae (including Atolla), with *Argyropelecus, Sternoptyx, Melanonus, Scopelogadus, Serrivomer, Cyclothone* and two small melamphaids and a few myctophids. Crustaceans were restricted to *Acanthephyra, Sergia, Gennadas,* and *Eurythenes*.

The next net (**Station no. 13673#2**) fished somewhat shallower, deliberately targeting *Systellaspis debilis* for the BBC, and euphausids for electrophysiology. On deck soon after 0300 it proved very useful for this purpose with numerous of both, as well as *Sergia* and *Sergestes*, a few *Acanthephyra* and small *Notostomus*. Fish were few but included many *Cyclothone* and two small *Melanocetus*.

The ship then set course for the lander station, some distance steam after the long day fishing.

**13th November 1999**

Recalled with usual efficiency the AUDOS lander was in board at 0800 and the ISIT lander prepared for deployment. This was over the aft end and released at 0924 (**Station no. 13674**) and operations then turned to RMT 25 fishing.

The first trawl (**Station no. 13675#1**) was deployed at 1000 and fished until just after 1800 at *ca*. 1500 m depth. The result was stunning, the star of the catch being a very large hairy angler fish (*Caulophryne pelagica)* which, after some deliberation as to whether if would fit in the kreisel or not, soon became the most photographed fish of the cruise, a steady procession filing past the tank to pay homage and take snaps. The third officer, Titus Owoso, dubbed the fish "the camcorder fish". Otherwise the catch contained several other anglers, (*Melanocetus, Chaenophryne*), as well as *Serrivomer, Platytroctes, Eurypharynx, Poromitra, Chauliodus, Melanonus*, and many hatchetfish. Cephalopods were represented by *Mastigoteuthis*, and *Vitreledonella*. Crustaceans by *Systellaspis, Acanthephyra, Notostomus, Sergia, Sergestes, Gennadas, Hymenodora, Meningodora, Gigantocypris, Cystisoma, Eurythenes, Eucopia, Gnathophausia* and others.

The second RMT (**Station no. 13675#2**), deployed only six minutes after the first was recovered, fished at a slightly shallower depth (700 m) and again specialised in angler fish, (*Himantolophus, Melanocetus, Oneirodes*) as well as *Chauliodus, Malacosteus, Stomias, Poromitra, Scopelogadus, Platyberyx, Serrivomer, Scopelarchus, Vinciguerria,* hatchets*, Opisthoproctus, Photostomias,* and a large *Borostomias.* The catch was also notable for many decapods, *Meningodora, Hymenodora, Acanthephyra, Systellaspis, Gennadas, Sergia, Sergestes,* one *Oplophorus, Parapasiphaea, Gnathophausia, Notostomus* as well as the cephalopods *Mastigoteuthis and Vampyroteuthis.*

Before the RMT 25 was recovered, a problem was reported in the engine room: a pipe taking cooling water from the engines had sprung a leak and repairs were required. Because the repairs required the isolation of one of the cooling pumps, operation on one engine only would be necessary, and thus our maximum speed would be reduced. In view of this a decision was made to abandon the next RMT deployment to enable us to return to the lander site at an engine speed of only 60 RPM, and much reduced ship's speed and retrieve the lander on schedule. Repairs to the pipe should be effected during the day tomorrow and no further interruption of the work schedule was anticipated.

On recovery the catch was unusually disappointing, containing numerous fish that appeared to have been caught very early in the trawl. Nevertheless, some interesting animals were recovered

**14th November 1999**

The ISIT lander was recovered without incident and the AUDOS readied for deployment. Whilst this was underway further information from the engine room indicated that the pipe was not repairable until Cadiz was reached. Until then we will be reliant on a patch which, because of the nature of the break, is only partially successful. Thus we are likely to have to steam on one engine with a maximum speed of 8 knots (150 rpm at the motor). In the hot water further south this might have to be further reduced. In view of this the departure time from the work area was brought forward from 0800 16th Nov to 1600 15th Nov. In consequence a decision was made to change the net deployment from OTSB to RMT25 in the course of today, with RMT 1+8 CCE tonight and, if time, tomorrow. This will provide most specimens for most people. Discussion with the lander team led to a decision to stay with the plan of an AUDOS deployment today, with an ISIT bungee deployment tomorrow as the last piece of science.

At 0918 the AUDOS (**Station no. 13676**) was in the water and soon below the surface. Preparations then began for rigging the RMT25. The wind remains force 4-5 but the swell is today more confused, with a long swell from the NW and a shorter swell from the north. The ship rolls gently under blue skies.

In the course of the day three RMT25s were shot, providing the last of the large material but in what proved to be a futile attempt to catch live *Pachystomias* for bioluminescence measurements, although we now have enough information to target particular species.

The first net (**Station no. 13677#1**) was recovered at 1654 after 1.5 hours at 1200m depth. This was notable for a single very large *Omosudis*, but also large *Cryptopsaras, Melanocetus, Serrivomer, Borostomias, melamphaids, Pachystomias, Platytroctes,* hatchets*, Sternoptyx, Stomias, Chauliodus, Flagellostomias* and one *Pachystomias*, unfortunately not alive. Once again *Vampyroteuthis* and Japatella were present as were *Atolla* and decapods including *Notostomus, Acanthephyra* and others as well as *Gnathophausia*.

The next net (**Station no. 13677#2**) arrived on deck in darkness in the late evening and was sorted under red light in the aft container to collect fish pineals. This contained a large *Borostomias, Eurypharynx, Opisthoproctus,* melamphaids*,* a juvenile macrourid, *Bonapartia, Sternoptyx, Malacosteus,* many *Platytroctes, Gonostoma, Chauliodus, Anoplogaster, Vinciguerria, Serrivomer,* and *Chiasmodon*. Cephalopods were well represented with numerous cranchids, *Vampyroteuthis, Mastigoteuthis,* and *Onychoteuthis.* Crustaceans included *Acanthephyra, Parapasiphaea, Gigantocypris, Sergia, Eucopia, Eurythenes*. As usual *Atolla* and *Periphylla* were present, as were several battered salps.

The final net (**Station no. 13677#3**) was again on deck in darkness and sorted under red light for the pineal study. This haul contained *Malacosteus, Aristostomias, Scopelarchus, Polyipnus* and other hatchets, a large *Diaphus* (a rare genus in this cruise) searsiids, *Chauliodus, Gonostoma, Opisthoproctus, Stylephorus, Vinciguerria,* and *Cryptopsaras*. Because of the depth the catch was bound in much jelly and few crustaceans (including *Acanthephyra, Sergestes, Gennadas*, and euphausids) had survived.

**15th November 1999**

After a steady steam back to the lander site the AUDOS was up as dawn broke and the ISIT prepared for a final deployment (**Station no. 13678**), this time with a bait (mackerel) to be placed and filmed on the sea floor. Once deployed the last trawl of the cruise, and RMT 1+8 CCE was readied and shot in an effort to obtain copepods for the BBC, and decapods for electrophysiological studies to proceed during transit to Tenerife. The RMT was shot soon after 10am and fished close to 500 m. On recovery it was notable for a total of 15 ceriatoid angler fish, all but one *Melanocetus*. Also in the catch were *Cyclothone, Chauliodus, Xenodermichthys, Stomias, Sternoptyx, Borostomias,* and *Malacosteus*. The only cephalopod was *Vitreledonella,* but crustaceans were well represented, both for the targeted copepods, but also for *Acanthephyra, Systellaspis, Sergestes, Gennadas, Notostomus* and *Sergia*: plenty for both the BBC and for the electrophysiologists.

At 1555 the ISIT lander was back on deck (in now somewhat heavy conditions, with a stiff wind) with five minutes to spare before the official end of science, the PES fish was brought in board, and the ship set passage for Tenerife. The ISIT video was spectacular with a convincing demonstration of spontaneous bioluminescence associated with feeding activity on the sea floor.

The end of science was celebrated with wine from the TLO, and a relaxed and sociable evening.

**16th November 1999**

As packing got underway a Science Debrief meeting wound up the science and laid out arrangements for demobilisation of the cruise, stowage arrangements for on board gear, chemicals etc. An emergency drill, synchronised with a passing sperm whale, gave the science party time to experience using different fire extinguishers, while Peter Shelton and Spencer Nyholm discussed action in the event of a chemical spill with ship's staff. Following the drill the science party as well as most of the ship's and RVS staff assembled on the boat deck for a cruise

photograph for which the old *RRS Discovery* life ring was brought out of storage. Fortunately sun and flat calm conditions made the activities easy.

**17th November 1999**

A morning debrief meeting was attended by IGP and the PSO from the science staff as well as the Master, C/O and RVS staff. Business was quickly completed.

In the evening, dinner ended with a fine speech and presentation by the Master, celebrating Peter Herring's last cruise on *Discovery* as an employee of SOC, and was followed by the PSO's traditional RPC. This ended only when a meteor shower diverted several of the participants from proceedings and onto the deck.

**18th November 1999**

The day spent cleaning laboratory space, packing equipment and preparing customs documents for those leaving in Tenerife, and checking that flight and hotel arrangements were in place. A supply of dry ice was manufactured from a $CO_2$ siphon cylinder in the evening in preparation for disembarkation of samples.

The ship is making good progress, and the repair to the cooling water pipe is holding out in engine room.

**19th November 1999**

0800 boat transfer Tenerife. The now familiar green boat, Beatriz-1, arrived somewhat late but, despite the marginal sea state, all samples, dry ice and scientists are unloaded without mishap.

Kley France (winch manufacturers) engineer on board, and Peter Mason (RVS) to work on winch systems.

Cleaning the laboratories continues.

**20th November 1999**

Weather much better than expected and progress north very good. As a result we have time for wire tests of winch.

Laboratory cleaning continues, and preparation of equipment for the transit steam and later unloading. Paperwork associated with the Cadiz port call accumulates.

In the evening Penny Howell's and Kevin Smith's birthdays are celebrated.

**21st November 1999**

In transit in continuing acceptable weather, and by late evening the lights of Cadiz are visible.

**22nd November 1999**

Cruise ends, Cadiz dock.

## 7 RVS Installed Equipment reports

### 7.1 Engineering/Instrumentation Report.

**1. 300KW Power Pack, 20T and 10T Cobra Unit.**

Only the 20 tonne cobra system was used.
During D242 a problem arose where there was no control to the winch, i.e. it could not be driven in either direction. A solenoid connection on hydraulic valve 57 was cleaned and the system gave no further trouble.
The first 24 deployments of D243 were ok. On 2/11/99 with 600m wire out, the fault appeared again. The power pack was shut down and restarted and the equipment recovered. No further problems were encountered until 5/11/99 when the system failed 3 times. On investigation and communication with SOC the following action was taken:-

a) The ATOS speed controllers from pumps 1 & 4 were changed over in the main control cabinet.
b) Inspection of the solenoid connection to hydraulic valve 57 revealed a split wire, this was remade and the connector cleaned.
c) Pressure switch 68 (main flow detect) was inspected and its operation checked ok.

Since then, another 28 deployments (over 10 days) have taken place without incident.
On initial deployment of the 13.4 mm trawl wire the cobra would not pay out, this was overcome by adjusting (increasing) the system working pressure.

**2. 10T Storage System, including 37KW Power Pack, Inboard Compensator & Diverter Sheaves.**

This system was not used. Component parts from the system have been used in the repair of the 20 tonne storage system during cruises 242 and 243.

**3. 20T Storage System, including 37KW Power Pack, Inboard Compensator & Diverter Sheaves.**

63 deployments of the Trawl Warp were completed, maximum wire deployed 11000 metres.
The problems encountered with this system at the beginning of the cruise and the subsequent delay in getting it operational resulted in 137.5 hours being lost to science.
During D242 a motor burnt out. This was replaced with a motor from the 10 tonne storage system 37 kW power pack. The burnt out motor was repaired during the Southampton port call.
Whilst in Southampton, 8000m of Aramid cable was removed from the system without any major problem arising. (ie. no faults or temperature cut outs were experienced.).
On passage to Tenerife, trial deployments of scientific equipment were performed. Again motor 1 (on the 20T 37Kw power pack) ran hot. The original rewound motor was refitted at sea. Once again a trial deployment of scientific equipment resulted in high temperature readings on this motor. Several days of investigations were carried out off Tenerife. The final outcome of these proceedings resulted in a hydraulics engineer (Eliot Beattie from Lawsons Engineering) coming out to the vessel. During the time he was on board (2.5 days), further investigations and system tests/repairs were carried out (see Eliot's report dated 5/11/99.) Although not 100%, the system was deemed sufficiently reliable/predictable to allow the cruise to go ahead. It was decided that the technicians would double up on watches and go on an 8on/8off shift pattern so that the system could be carefully monitored at all times.
The system was then used for the duration of the cruise for the number of deployments listed above without any other major failures.
At Tenerife a Kley France hydraulic engineer together with Pete Mason from SOC joined the vessel. System tests and trials are still continuing. A further report will no doubt appear in due course!

**4. Millipore Water System.**

The system has been in continual use for the cruise on a daily basis, no problems arose.

## 5. Radio Nuclide Container Laboratory.

Used for the duration of the cruise as a " dark " room for work with scientific specimens. On initial installation of the container the air conditioning unit had tripped due to low water pressure. To reset the unit involves the removal of the front panel of the electrical control box. A modification has been carried out in the past on similar systems to enable this action to be carried out a lot easier. ie by fitting of an access plate on the front panel.
As both doors of the unit were in continuous use a complaint has been raised over the accessibility of the push handle to open the main lab door. This is because of the positioning of the laminar flow hood which protrudes across the door covering approx half the handle making it difficult to push directly without risk of injury.

## 6. 10ft Container.

This container , due to its proximity to the net catches , was used as a sorting "dark" room where catches were divided amongst the scientific parties as soon as landed on deck . 240 Volt electrical supply was wired into the container at the beginning of the cruise. No problems were encountered.

## 7. Seametrix System.

The system started the cruise without the following functions :- wire out, wire veer/haul rate, wire out reset, audible alarms, and alarm acknowledge/cancel. The tension readings and logging to the ships computer were ok. A new encoder had recently been fitted.
During the passage to Tenerife, the output from the replacement encoder was checked and found to be ok. It was suspected that the problem lay in the interface electronics and/or the pc. Spares were requested for the Tenerife port call.
Both system interfaces were checked and appeared to be working ok. A malfunctioning relay on the pc I/O board suggested possible problems in the Tower unit/pc. The I/O board in the pc was replaced with no effect, original refitted. The 37-way ribbon cable between the I/O board and the interconnection pcb in the system power unit was removed. This restored wire out and veer/haul rate. No further repair work was attempted owing to the lack of appropriate spares. The system was run like this for the remainder of the cruise. Spares were requested for the 2nd Tenerife port call! This time they arrived.
On passage to Cadiz, the system power unit and Tower wiring were checked. No 24v supply was observed on TB1. The 24v psu in the system power unit was found to be u/s and was replaced with one from the Challenger unit. This restored the 24v but still no joy with alarms! No 12v supply was found on the interconnection pcb, This was removed and inspected. The track around the 12v zener was burnt out. This pcb was replaced again with one from the Challenger unit. Once more the 24v supply was dragged down! All connectors (except the power connector J4) to the board were removed. This restored the 24v/12v. It was found that i/p connector J5 (watchdog monitor) was dragging the unit down. This was left disconnected and the system was successfully run up. Alarms, ack/cancel and wire reset functions ok. Wire out, tension, and computer logging ok.

## 8. Simrad EA500.

The Simrad EA500 echo sounder was run for the duration of the cruise using the PES fish transducer and the beam steering unit. User equipment was connected to the single element transducer connection. No problems were encountered.

## 9. RDF System.

A rotary antenna was installed on the monkey island. Modifications to the installation wiring were completed during the cruise. It is now possible to simply plug-in/uplug the system equipment and install/remove from the ship. The system was used for detecting and locating landers on the surface.

**Rhys Roberts, Kevin Smith, Phil Taylor, Darren Young.**

## 7.2 Computing Report

**1. Level ABC Data**

During this cruise, the following instruments were logged using the RVS ABC Computer System:

Ships Gyro - giving heading information

Chernikeeff two component EM-log - giving fore/aft and port/starboard speed through the water.

Trimble 4000 GPS - giving position and speed over the ground.

Ashtech attitude GPS system - giving position and ship roll and pitch information.

Ashtech GPS/GLONASS system - giving position and speed over the ground. This system suffered from a fault when it was receiving differential corrections, so that a longitude which should have been to the west, actually came up as being to the east. It also seemed to be around 13 minutes slow with its time stamps.

Simrad EA-500 echo sounder - giving bathymetry. This wasn't switched on all the time, as the fish was also used to release landers.

**2. Data Processing**

Navigation was processed using the gyro, log and Trimble GPS using the Level C *relmov* and *bestnav* programs to give positions every 10 seconds.

The bathymetry data was processed using the Level C *prodep* in order to correct for the approximate local speed of sound in water (using Carter Areas).

**3. Miscelaneous**

The Level B system suffered from a Black Hole condition (thought to be tape related) on 15th October. Luckily the Level C alarm alerted me, and only 5 minutes of data was lost from 99 288 07:37:54 to 99 288 07:42:19.

There was another Level B crash on 2nd November, but this time only 15 seconds of data was lost between 99 306 09:46:52 and 99 307 09:47:07.

The Seametrix winch monitoring system would normally also be logged, but its SMP (Ship Message Protocol) output to the Level B does not appear to be working at the moment.

At around 8:30pm on the 5th November, a segment of the ship's computer network covering the computer room, plot, photo lab and main lab developed a fault. Investigations were carried out until sometime after midnight with no positive results. The next day the network wiring diagrams were consulted and slowly the whole of the wiring which formed the segment was blocked off using a network terminator and tested. This had the effect of fixing the fault, so it is possible that the fault could have been some slight corrosion on one of the BNC connectors. The ABC segment of the network was unaffected, and so data logging carried on with no interruptions. When *Discovery* is next in Southampton this co-ax (AKA "10base2") based network segment is due to be replaced with twisted pair (AKA "10baseT") networking which has the advantage that a faulty piece of cable going to one computer, cannot affect others on the network.

Station data were input to the Level C *biostn* (Bio-Station) program to be later printed out in the IOS Cruise Report format. Unfortunately the output format is not quite correct, and this will be reported back to RVS and hopefully the output format will be corrected.

Charts of deployment positions for the AUDOS and ISIT landers, and RMT1+8, RMT25, OTSB and Oxfam nets were also produced, and an A4 whole cruise track will be produced at the end of the cruise.

Final navigation data and corrected bathymetry data will also be put into ASCII format using the Level C *mutli* program, and then copied to Zip disk.

**Paul Duncan**

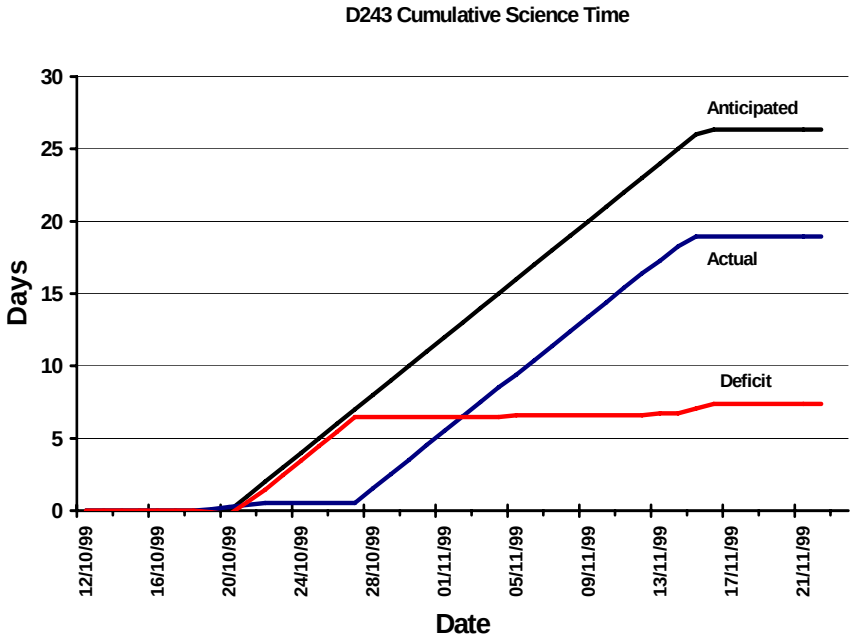
## 8: Cruise Time budget

Within the total time at sea during D243, or during any cruise, some fraction of the total time at sea must be allocated to tasks that are scientifically not directly productive but which are, nevertheless, essential. Passage time to the work site is in this category. For the purposes of this section I have included such periods with the times when the ship's activities are directly dominated by scientific procedures, such as trawling, under the category of "science time". On any cruise, one hopes to maximise science time.

The potential science time at the beginning of a cruise is reduced by such things as bad weather (e.g. high winds), problems with the scientific equipment (e.g. failure of acoustic net controls), problems with the ship itself (e.g. engine overheating), and problems with the installed gear (e.g. winch failure). These are separated below and the total science time show in the figure.

Bad weather did not reduce science time at all during D243 and only once was the order of work altered in deference to the weather. This was despite the fact that the weather was worse then anticipated. The "average" for October/November in the region, according to the Africa Pilot, is force 3 or 4, but we encountered weather substantially worse than this, up to force 8. We had almost no problems with scientific equipment, and none that resulted in complete disruption of scientific activities. We also had almost no down time due to problems with the ship and in total lost only *ca*. 11 hours in this way: an excellent record. We did, however, loose a substantial time (7.4 days out of the total anticipated of 26 days) to the failure of the winch system early in the cruise. Although repairs were eventually made, the work ate into time that would otherwise have been spent on productive scientific work and nearly 30% of the potential science time of the cruise was lost to winch repairs. Clearly this affected the productivity of the cruise.

The cumulative expected science time, the actual time, and the deficit are shown below.



## 9 scientific investigations and allied work

### 9.1 Lander Deployments.

Two autonomous lander vehicles from the University of Aberdeen were used during this cruise for *in situ* studies of biological phenomena on the sea floor, the AUDOS and ISIT landers. Both landers were operated in a very similar way, being deployed by streaming buoyancy over the stern of the ship while going slow ahead. The lander itself was then lifted into the water using one of the aft cranes through the 'A' frame and releasing with a Tow 10 Boss quick release. Each lander was equipped with twin MORS acoustic releases, and for recovery commands were sent using the University of Aberdeen ARCADE system. Altogether 22 lander deployment and recoveries were achieved with no loss or failure of equipment except for one MORS RT unit which leaked. Failure of this unit was detected on deck. A constraint on the work was that there was only one current meter available between the two vehicles which meant both landers could not be deployed simultaneously.

**AUDOS. (Aberdeen University Deep Ocean Submersible)**

The aim of these experiments was to identify the dominant deep demersal scavenging fish species in the area of the Cape Verde tropical upwelling, to measure their times of arrival and departure at an artificial food fall and to track the movements and swimming speeds of these fishes as they depart from the bait source. These data are to be compared with studies from elsewhere in Atlantic Ocean and the Pacific Ocean. The AUDOS vehicle is deployed with a downward-looking time-lapse camera (800 frames at 1min intervals) suspended 2m above the sea floor. Bait, mackerel and a measuring scale are placed on the bottom within the centre of the field of view of the camera. Ektachrome 200 ASA film was used and short segments were developed on board, (E6 process) for checking function of the system and for a preliminary examination of species present. The AUDOS also carries a special short base-line sonar capable of tracking miniature Code Activated Transponders (CATs) which were deployed in bait bags so that they can be ingested by scavenging fishes.

One test deployment of AUDOS was carried out off the Canary Islands during the early part of the cruise. The rest of the work was focussed on two sites, one at ca. 3200m depth on the Cape Verde Terrace (7 deployments) and a second at 4040m between the Mauritania and Kayar Channels to the South (3 deployments). Plans for studies over a wider bathymetric range were not possible owing to constraints resulting from time lost early in the cruise

**Operation of the vehicle.**

The AUDOS was deployed with one large syntactic buoyancy block, a Dahn buoy, and two new CRP "egg" floats as in Fig 1. Except for the Dahn buoy no glass spheres were used. For deployment, the syntactic block was lifted using a cable from the auxiliary deck winch, over a block on the A'frame and a slip hook for release.

**Photographic Data**

From each AUDOS deployment two short segments of film were processed on board. This allowed for the preliminary examination of the main scavenging fish species present in the two main deployment sites.

The Macrourid *Coryphaenoides (Nematonurus) armatus* appears to be the dominant scavenging species attracted to the AUDOS bait. It was observed in photographs from every deployment (including the test site off the Canary Islands). Often a single frame showed up to four individuals. The presence and identification of this fish species were confirmed by specimens caught in the OTSB net on both deployment sites.

Photographs from the Cape Verde Terrace revealed the presence of another species of fish. This is thought to be the zoarcid *Pachychara abyssum* because of the depth and its position in the water column as well as some typical body features observed on the photograph. There

were no zoarcids in the OTSB catch to confirm its identification. The holothurian *Enypniastes* was also observed in photos from two deployments.
On the photographs from deployments in the abyssal plain another two fish species were identified, *Barithrites iris* and *Histiobranchus bathybius*. Both identifications were confirmed by specimens caught in the OTSB net.
Photographs and body lengths of the specimens caught in the OTSB net were taken to facilitate the observation of the AUDOS photographs.

**Tracking Data**

The short base-line sonar situated on the AUDOS lander interrogates CATs within acoustic range. Interrogations consist of a twin acoustic pulse separated by a programmable delay. A CAT detecting a valid interrogation code responded with a single return pulse, which was detected by each of the 3 hydrophones situated on the AUDOS lander. These responses were logged on-board then downloaded and processed once the vehicle was recovered. Two dimensional track reconstruction is to be completed post cruise. For ship board analysis the maximum tracking range for each successful track was determined. Each track lasted several hours and contained an average of 200 data points.

**Table 1: Fish tracking results**

| Station | No. of fish tracked | Tracking ranges | Comments |
|---|---|---|---|
| 13635 | 3 | 300m,602m,440m | |
| 13639 | 3 | 667m,629m,550m | 1 CAT fell off |
| 13644 | 1 | 250m | 4 CATs fell off, 1 landed close to AUDOS, picked up by a fish |
| 13646 | 2 | 250m,607m | |
| 13650 | 4 | 300m,290m,450m,725 m | |
| 13654 | 4 | 443m,270m,270m,270 m | |
| 13663 | 3 | 777m,605m,806m | 1 CAT remained on the cross |
| 13666 | 3 | 640m,477m,679m | |
| 13668 | 2 | 378m,444m | 4 CATs fell off, 2 landed close to AUDOS, picked up by a fish |
| 13671 | 3 | 400m,550m,789m | |
| 13676 | 3 | 319m,563m,600m | |

A total of 31 fish were successfully tracked to a mean range of 495m (806m maximum), which represented a 70% success rate.

**ISIT (Intensified Silicon Intensified Tube) Lander**

This lander carries a high sensitivity ISIT video camera (Simrad, Aberdeen) and the aim was to investigate bioluminescence in the deep sea benthic boundary layer. The ISIT camera is linked to a command and digital video tape recording system in a separate housing. The system can be programmed using an on board real time clock to power up/down the camera, start/stop recording and on/off an incandescent 20W light. Thus for a typical deployment, after a delay allowing deployment and descent to a depth away from sunlight that would damage the camera tube, the camera was switched on and allowed to warm up for 60s before the tape recorder was activated to observe bioluminescence, finally at the end of the sequence the light was switched on for 10-30s to observe any biota. A low intensity blue LED could also be activated to provide a calibrated light source within the field of view. A current meter

(Sensortec) provided data on depth, temperature, current speed and direction at 1min intervals. The experiment was configured in three different ways:

**Free-fall "Bungee" mode.**

The camera was fixed looking downwards beneath the lander frame. At a distance of 50cm from the lens, a mesh screen (16mm x 8mm mesh pitch) was placed filling the entire field of view of the camera. Video recording began at 500m depth and was repeated at regular intervals until impact with the sea floor. With a descent rate of $34 m.min^{-1}$, $6.46 m^3.min^{-1}$ of water were filtered through the mesh screen. Biota were stimulated to luminesce as they passed through this screen. There was a clear decrease in frequency of luminescent events with depth but with an increase in the last few meters before landing. Three such vertical profiles of bioluminescence were recorded at the 4046m deep station.

**Benthic Flow mode.**

The camera was mounted horizontally on a turn table with the centre of the lens 57.5 cm above the sea floor. A mesh screen (1.5 x1.5mm pitch) was placed at a distance of 50cm filling the entire field of view of the camera. The camera was linked to the current meter so that it was rotated to face into the bottom current at all times once the lander had reached bottom. Both at 3200m and 4000m depth luminescent biota were observed to hit the mesh either swimming actively or driven by the tidal currents of the benthic boundary layer. Current velocities of up to $9 cm.s^{-1}$ were recorded with a mean of 4-5 $cm.s^{-1}$.

**Baited mode.**

The camera was fixed looking downwards focussed at a distance of 80cm to view the sea floor. A bait, comprising mackerel and some prawns was placed on a steel bar so that when the lander arrived on the sea floor it was in contact with the sediment. About 0.5h after landing on the sea floor luminescent events were observed on the bait. These increased in intensity and frequency and were associated with the presence of the prawn *Plesiopenaeus armatus* and the grenadier fish *C.(N.) armatus* although neither of these animals themselves are thought to generate light. By two hours after arrival on the sea floor luminescent events were quite frequent suggesting that carrion falls on the sea floor may be advertised in such a way. Only one baited mode deployment was carried out, at 3200m.

All three modes of operation of the ISIT lander provided evidence for bioluminescence in the benthic boundary layer at depths of 3000-4000m. This is a new discovery clearly achieved during this cruise; previous work on bioluminescence has been largely confined to depths less than 1000m owing to limitations of ROVs and submersibles generally used for such studies.

**Funding.**

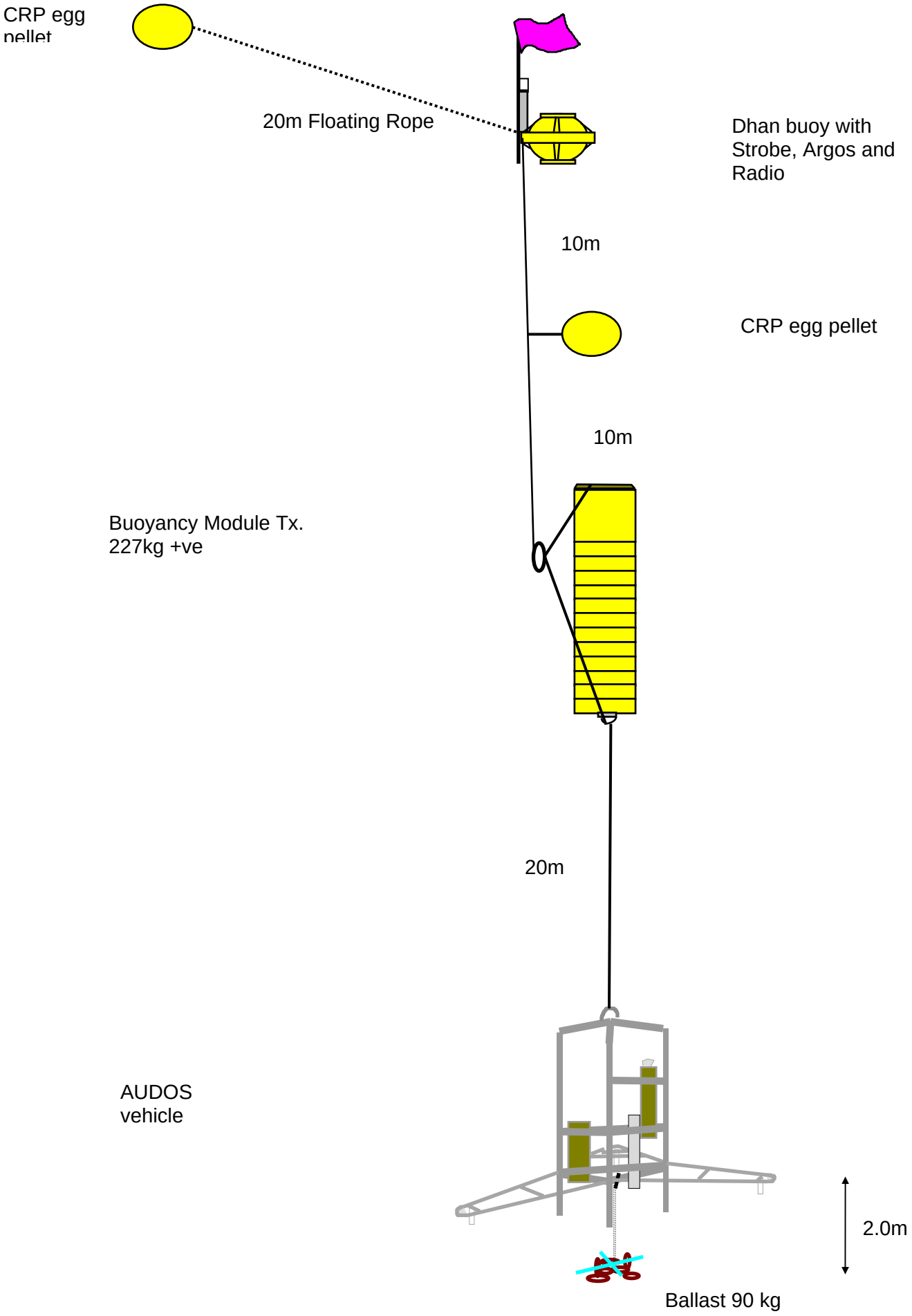
Immediate funding for ISIT hardware and the rotating mechanism of £12,000 was provided by a NERC grant awarded to P. J. Herring, J.C. Partridge, and P.M.J. Shelton. The ISIT camer was provided by P.J.Herring. The total cost of the landers is *ca* £100,000 each and was provided by EU MAST III contract ALIPOR (CT950010). Further costs of participation in the cruise and running costs of the landers ca. £20,000 was from the University of Aberdeen.

**Objectives met.**

The scientific objectives were largely achieved but the loss of working time meant that work was focussed on just two stations. Originally a transect of 10 benthic stations was envisaged, later reduced to multiple deployments at three stations of differing depths, further curtailed to two stations. Only one baited ISIT station was possible.

**I.G. (Monty) Priede, Phil M. Bagley, Susannah Way, Camila Henriques**

**AUDOS Mooring**



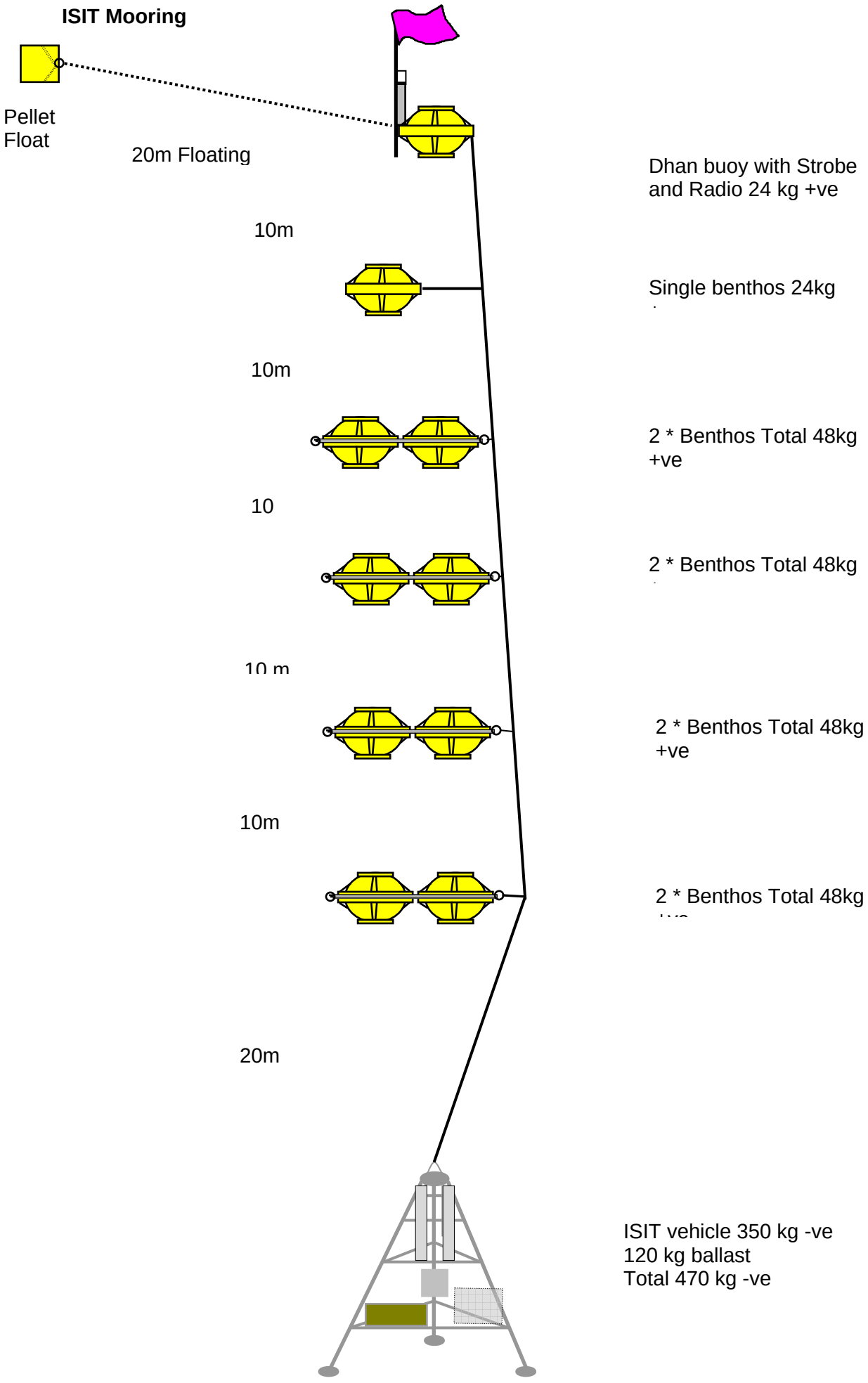

ISIT Mooring
Pellet
Float
20m Floating
Dhan buoy with Strobe
and Radio 24 kg +ve
10m
Single benthos 24kg
10m
2 * Benthos Total 48kg
+ve
10
2 * Benthos Total 48kg
10 m
2 * Benthos Total 48kg
+ve
10m
2 * Benthos Total 48kg
20m
ISIT vehicle 350 kg -ve
120 kg ballast
Total 470 kg -ve

**Table 2**

*AUDOS deployments D243*

| *Deployment* | *Date* | *Station* | *N* | *W* | *Depth* | *Comments* |
|---|---|---|---|---|---|---|
| 1 | 21-Oct-99 | 13635 | 27°43.73' | 16°59.03' | 3351 | Camera BST. Very slow descent. *Coryphaenoides armatus* |
| 2 | 28-Oct-99 | 13639 | 17°40.84' | 20°00.26' | 3254 | Camera GMT. No current meter. *C.armatus* |
| 3 | 30-Oct-99 | 13644 | 17°30.27' | 20°15.00' | 3244 | Current meter back on. *C.armatus* |
| 4 | 1-Nov-99 | 13646 | 15°00.10' | 20°29.95' | 4046 | *C. armatus* |
| 5 | 3-Nov-99 | 13650 | 15°01.72' | 20°30.06' | 4038 | *Barythrites iris, C.armatus* |
| 6 | 4-Nov-99 | 13654 | 14°58.86' | 20°30.01' | 4046 | *Histiobranchus bathybius, C.armatus* |
| 7 | 9-Nov-99 | 13663 | 17°40.08' | 20°00.10' | 3258 | |
| 8 | 10-Nov-99 | 13666 | 14°58.86' | 20°30.01' | 3220 | *Enypniastes*, Zoarcid, *Munidopsis crassa*(?) |
| 9 | 11-Nov-99 | 13668 | 17°49.63' | 19°56.92' | 3198 | *Enypniastes, C. armatus* |
| 10 | 12-Nov-99 | 13671 | 17°49.99' | 20°03.33' | 3208 | *C. armatus* |
| 11 | 14-Nov-99 | 13676 | 17°54.01' | 20°03.8' | 3199 | |

**Table 3**

*ISIT deployments D243*

| *Deployment* | *Date* | *Station* | *N* | *W* | *Depth* | *Comments* |
|---|---|---|---|---|---|---|
| | 14-Oct-99 | 13630 | 44°08.20' | 10°43.27' | 4939 | Wire test |
| 1 | 19-Oct-99 | 13632 | 26°50.43' | 18°12.45' | 3730 | Rotator. No data |
| 2 | 20-Oct-99 | 13634 | 27°16.85' | 17°44.17' | 3690 | Rotator. One hour delay only |
| 3 | 28-Oct-99 | 13640 | 17°41.40' | 20°00.15' | 3251 | Rotator. Three hour delay |
| 4 | 29-Oct-99 | 13642 | 17°40.28' | 20°00.46' | 3256 | Rotator. Three hour delay |
| 5 | 2-Nov-99 | 13648 | 15°00.14' | 20°30.096' | 4046 | Rotator. Three hour delay |
| 6 | 4-Nov-99 | 13653 | 14°58.96' | 20°29.82' | 4046 | Test bungee |
| 7 | 5-Nov-99 | 13656 | 14°59.86' | 20°29.61' | 4046 | Bungee |
| 8 | 6-Nov-99 | 13658 | 15°00.16' | 20°30.01' | 4046 | Bungee |
| 9 | 7-Nov-99 | 13661 | 15°00.08' | 20°30.11' | 4048 | Rotator. Three hour delay |
| 10 | 13-Nov-99 | 13674 | 17°51.86' | 20°03.58' | 3201 | Rotator. Three hour delay |
| 11 | 15-Nov-99 | 13679 | 17°57.32' | 20°03.15' | 3199 | Baited bungee |

## 9.2 Trawl programme

The midwater sampling programme revolved around the RMT system. This comprised the RMT 1 + 8 closing net system, with acoustic control and monitoring, fished either with standard buckets or with a closing codend which was also operated by acoustic command. For RMT 1+8 CCE deployments fishing depth (d) was related to meters of wire out (w) as w=0.5w +60. An RMT 25 net (kindly loaned by the British Antarctic Survey) was also employed on many occasions. This net was operated in the open mode and fitted with a standard codend bucket and liner. It was hauled in at speeds of either 10 or 15 m $min^{-1}$ in order to minimise damage to the catch. The net monitor was not used with the RMT 25 and the depth of the net was therefore estimated (on the basis of previous monitor data) as half the length of wire out.

The RMT 1+8 was used three times close to Tenerife, largely to test the winch system before moving south, and there were 40 tows with the closing codend and 15 of the RMT 25. Both nets worked well, with no significant handling problems. A few minor problems with ball valve monitoring and sealing on the closing codend were easily rectified. For successive tows with the RMT 25 it was brought to the stern and the codend bucket recovered on a handling line for collection of the sample. The net was then redeployed for the next tow.

The catches were generally very large and diverse, with the regular inclusion of very large and soft *Pyrosoma* and salps, particularly at the more northerly position. Despite its physical inconvenience it probably helped to maintain the catches in excellent condition, though it limited the flow rate through the closing codend liner. The use of the RMT 1+8 with the closing codend, and a subsequent “dark” sort under red light in the deck container, provided a regular supply of live material for neurophysiology and dark-maintained material for visual pigment and pineal pigment studies.

Four tows were made with the OTSB14 otter trawl in depths of 3200-4100m. On the first deployment the doors locked, resulting in a very small sample, but the other three were all successful, achieving large samples of fish and invertebrates.

**Ben Boorman, Dave Edge, Peter Herring, Nigel Merrett**

## 9.3 Studies on trawl Samples

### 9.3.1 Structure and function of compound eyes and photophores in mesopelagic crustaceans.

The present study represents part of an ongoing programme to investigate the ways in which crustaceans both detect and produce light within the oceanic environment. The oceanic light climate is highly predictable with respect to spectral distribution, angular distribution about the vertical axis and intensity changes with depth. These factors should be reflected in the designs of both the visual systems and the light emitting organs (photophores) of crustaceans. Superimposed upon the environmental parameters are behavioural factors: some shrimps undergo substantial dawn and dusk vertical migrations and others do not. With respect to sunlight, little remains below 1000 m, below that depth only bioluminescent light remains. In the current study animals were mainly collected from depths of 200 m to 1200 m. The transition from an environment dominated by sunlight to one dominated by bioluminescence should result in significant differences in the designs of eyes and of photophores. Detecting such differences was one of the major objectives. With respect to eyes we are interested in both their ocular anatomy and their retinal physiology. Two objectives were set: (i) to obtain preserved specimens of the eyes

of representative species for comparative studies of eye structure and (ii) to cooperate with Rachel Ince and Julian Partridge in obtaining physiological records from the eyes of species of known retinal morphologies. Eyes preserved for anatomical study were preserved in aldehyde fixatives for later post-fixation and embedding. Some of the material will be wax embedded and will be used for light microscope studies, the majority will be treated in osmium tetroxide and resin embedded for electron microscopy. Our previous studies have shown that rhabdom morphology varies according to depth distribution and, in upper mesopelagic species, on the the position of the rhabdom within the eye. Thus, upwardly-looking rhabdoms in species such as *Oplophorus spinosus* have boat-shaped rhabdoms that are pointed at both ends and which are square in cross section. They are also spacially separated from each other. Such rhabdoms are well adapted for acute vision. Downwardly-looking rhabdoms have a much more elaborate morphology and they often interdigitate so that adjacent rhabdoms are not optically independent. This arrangement maximises sensitivity. In addition to differences in gross morphology, the internal organisation of the rhabdom varies from species to species. Most but not all species have two components to the rhabdom - a small distal region formed by microvilli from receptor cell R8 and a larger proximal region formed by receptor cells R1 - R7. These are known to be sensitive to blue and green parts of the spectrum respectively. Variations in the proportions of the rhabdom formed by each of the two elements can vary considerably. Species such as *Systellaspis debilis*, *S. cristata* and *S. braueri* all possess large R8 rhabdoms. Others such as *Acanthephyra pelagica* and *A. purpurea* have miniscule R8 rhabdoms. *Notostomus auriculatus* lacks an R8 cell altogether. A major objective of the present investigation is to investigate rhabdom morphology to see how rhabdom design varies with depth and to see whether there is any logical explanation for the variations in the relative sizes of R8 that are found between species. In addition, studies of rhabdom gross morphology and volume will be carried out. Some species near the bottom of the photic zone have reduced eyes. These include species of *Hymenodora* and specimens were taken for ultrastructural analysis. On the present cruise it was noticed by Dr P.J. Herring that specimens of *Meningodora miccyla* have hexagonal facets whereas most other oplophorid shrimps have a square packing arrangement characteristic of the reflecting superposition eye type that is typical of this group. Hexagonal facets were also found in *M. mollis*. *M. vesca* was found to have a less regular facet pattern but with square packing over much of the eye. Thus, within the genus there appears to be a transition from species with reasonable optics to those without. Ultrastructural studies on these specimens will be carried out to see how such modifications are reflected in internal anatomy.

With respect to photophores, we have already described details of the anatomy and modes of rotation in the cuticular photophores of the oplophorids *Oplophorus spinosus* and *Systellaspis debilis.* Comparative studies will now be carried out on the photophores of penaeid shrimps. Specimens were obtained from two species of *Gennadas* and from three species of *Sergia*. In addition, photophores from two species of euphausiid were obtained to complete the study. The work will include: an investigation of photocyte structure including comparisons of their paracrystalline bodies with those of the oplophorids, the organisation of reflecting and screening pigment cells and mechanisms involved in photophore tilting where this occurs. During the course of the study over 100 eye and photophore specimens were obtained. In addition, over 50 whole shrimps were preserved for future comparative investigations of brain anatomy in mesopelagic shrimps.

With respect to physiological studies, an agreed stimulus programme was discussed with Rachel Ince prior to the cruise involving two types of stimulus. These were square pulses and sinusoidal oscillations of an LED light source. V/logI curves were used to find the linear part of the intensity/response curve. The aim was to obtain information on the frequency response properties of the eyes of mesopelagic selected shrimps. My main input on board was to help set up the recording apparatus which was then used by Rachel Ince to obtain responses from a number of species of oplophorid.

The study was supported by a NERC project grant to PMJS part of a joint research grant held by Peter Herring, Julian Partridge and PMJS.

Estimated cost of participation in cruise and working up samples: £4,000

**Peter Shelton**

### 9.3.2 Electrophysiology of crustacean eyes

The purpose of participating in the cruise was to carry out extracellular electrophysiology on the compound eyes of mesopelagic decapods in order to determine the temporal resolving power of the eyes of species of decapod of differing depth distributions. This report describes work undertaken in collaboration with **Julian Partridge** and **Peter Shelton**.

Deep sea species are of particular interest because they inhabit an environment in which the spectral composition, angular distribution and intensity of light are strongly regulated by depth. The response of the visual system is strongly correlated with the light limitations of the marine environment. Those animals inhabiting the deep sea are light (photon) limited and therefore their eyes are concerned with maximizing photon capture and hence increasing sensitivity. Increased sensitivity of the eye is gained at the expense of temporal resolution. The photoreceptor response slows in order to increase the amount of time during which it can integrate photon capture. Due to this one would expect to see that mesopelagic decapods have 'slow' eyes in comparison with coastal/estuarine species which inhabit environments in which there is more light available. To test the hypothesis that mesopelagic decapods have eyes with slower temporal characteristics than inshore decapods I will repeat the electrophysiology on inshore decapod species and so collect comparative data. Also, one would expect to see differences in the temporal resolving powers of the eyes of shrimps from shallow depths in the ocean and those from greater depth, as light intensity in the ocean decreases predictably with depth.

Bioluminescence in the ocean is the only source of light available at depths greater than 1000m. Therefore it becomes very important in terms of visual stimulation. For mesopelagic shrimps it is also important, as there are a number of species that produce bioluminescence, for example, *Systellaspis debilis*. This species produces a bioluminescent spew when threatened, but also possesses photophores which are used in communication. However, the deeper living relative, *Systellaspis braueri*, is non-luminescent. It is the speed of the response of the photoreceptor which determines the quality of the eye's neural image of a moving environment. If the response is slow, spatial detail will be blurred by movement and visual information lost. So in that respect, it will interesting to look for differences in the speed of the eye not only in relation to depth distribution, but also in relation to the ability to bioluminesce. Those species of decapod which are bioluminescent may require a greater photoreceptor response speed in order that they can efficiently detect signals from other organisms. This will then potentially provide information about the interactions between organisms in the deep-sea environment.

Four species of decapod were used for this study, *Systellaspis debilis* (bioluminescent), 9 individuals, *Oplophorus spinosus* (bioluminescent), 2 individuals, *Notostomus spp*. (non-bioluminescent), and *Acanthepyra* spp (non-bioluminescent), 5 individuals. These were caught in a light and thermally protected closing cod-end used with the RMT 1+8 net. Providing the eyes were in good condition the live animals were then maintained and used for electrophysiology. Therefore all data were obtained on board ship and will be analysed at a later date. There was an abundance of decapod species available from the mid-water trawls. Those specimens used for electrophysiological recordings were preserved. Measurements

of eye size, total length and standard carapace length were recorded prior to preservation.

Recording from the eye. The animal was secured by the carapace to an aluminium holder, and immersed in a chilled water bath (7°C) with eyes above the water level. An insulated tungsten microelectrode was inserted just below the corneal layer of one eye. A second, platinum, electrode was inserted into the water bath as ground. The preparation was carried out under dim-red light. The response was amplified using a Neurolog DC Amplifier and recorded on an oscilloscope. Data was then transferred from the oscilloscope to a PC using SP107 software and an RS232 interface. Two forms of light stimulation were used. The first involved stimulating the eye with pulses of light varying in duration from 1.4ms-250ms. This stimulus was delivered to the eye via a fibre optic placed 4mm from the eye. The light source was either a blue (470nm) LED or a green (505nm) LED. The intensity of the stimulating light was controlled using absorptive neutral density filters. The second stimulus was sinusoidal, varying in frequency from 0.1-20Hz. The light from the blue LED was passed through an interference filter at 490nm, the light from the green LED was passed through an interference filter at 520nm. Again, the intensity of the light was controlled using absorptive neutral density filters. After placing the animal in the water bath and inserting the electrodes, a test pulse of 1.4ms duration and ND4 was used at 30min. intervals until the response had stabilised. The test pulse was then used throughout the experiment to ensure the eye wasn't light adapting. Periods between stimuli were as long as one hour when stimulating sinusoidally. This presented a problem in terms of time and as a result only 16 animals were tested during the cruise.

The collected data from the sinusoidal stimulation will be used to construct frequency response curves, plotting both amplitude and phase information from the response. The pulse data will be used to study the effect of pulse duration and stimulus intensity on the time to peak of the response. Stimulating the eye of *Systellaspis debilis* using the green LED shows a difference in waveform of response to stimulation with the blue LED. This suggests the presence of two different photoreceptors. Stimulating the eye of *Notostomus* and *Acanthephyra spp*. With the green LED did not produce a change in the shape of waveform.

Funding for this work was provided by NERC, the grant (GR3/B1212) awarded to Peter Herring (Southampton), Julian Partridge (Bristol), and Peter Shelton (Leicester), the purpose of the grant being to study visually mediated interactions in the deep-sea environment.

**Acknowledgements:**

I would like to thank the ship's company for making it easy to work efficiently, and for making the cruise such a pleasurable experience. I also thank Dave Edge for technical support, and Tammy Frank for her help and input with respect to the electrophysiology protocol.

**Rachel Ince**

### 9.3.3 Visual Physiology of midwater crustaceans

Electrophysiological studies were conducted on various species of midwater organisms that came up alive in the closing cod-end of the RMT 8 net. Parameters examined were spectral sensitivity, absolute sensitivity, and temporal resolution. This is part of an ongoing study to determine how the visual ecology of midwater crustaceans, particularly those that vertically migrate, is correlated with their distribution in the water column. Vertical migrators (organisms that live in deep water during the day and migrate up at night to surface waters) are of particular interest because they are close to the base of many oceanic food webs, with many

species of both adult and larval fish relying on these migrators as their major source of food. Previous *in situ* studies have demonstrated that these migrators not only are staggered with respect to their depth distributions during the day, but also undergo a staggered migration pattern, with some species starting their migrations earlier than others. Therefore, these shipboard based studies were undertaken to determine whether the species that are found deeper in the water column during the day, and/or start their migrations later are actually more sensitive to light than shallower species. In addition, studies on the temporal resolution of their photoreceptors (how well their eyes track moving objects) were continued, to determine the correlation between this parameter and the bioluminescence of their predators or prey.

27 experiments were conducted on oplophorid, euphausiid, penaeid and sergestid crustaceans, with the following results:

1. Shallow water crustaceans possess a circadian cycle of sensitivity in the photoreceptors, with their receptors becoming more sensitive to light at night. There has been some speculation as to whether this occurs in vertically migrating midwater crustaceans as well, with a pre-sunset increase in sensitivity aiding them in detecting the light cues that trigger the onset of their migrations. Studying this phenomenon requires monitoring the sensitivity of the eye by giving it a test flash of set wavelength and intensity once every 30 minutes for 48-72 hours. Normally I would not utilize valuable shiptime for such a long-term experiment. However, due to problems with the trawl winch that occurred after the first trawl, which restricted access to new species for several days, I had the perfect opportunity to run one of these experiments on the vertically migrating oplophorid *Systellaspis debilis*. This experiment ran for 56 hours, and demonstrated that in this species, there are no cyclic changes in sensitivity

2. Previous studies have demonstrated that, with the exceptions of several species of oplophorid crustaceans, the spectral sensitivities of all other midwater crustaceans peak around 490 nm, which is blue-shifted from those of their shallow water relatives. On this cruise, I measured the spectral sensitivity of several species of euphausiids, oplophorids and sergestids, with no unusual results. However, one species of penaeid, *Plesionika* sp., appears to have two visual pigments, and preliminary results indicate that one of these might be red shifted, peaking around 540 nm. The chromatic adaptation experiments gave puzzling results, without a clear answer as to whether one of the pigments really has such a long wavelength peak. However, the change in response polarity at different wavelengths clearly indicates that two classes of receptor cells are present, and hopefully MSP studies by Julian Partridge and Rachel Ince on the visual pigments of these unusual photoreceptors will give more definitive answers.

3. Temporal resolution is inversely related to sensitivity; eyes adapted for greater sensitivity tend to have lower temporal resolution. The midwater crustaceans in this study come from a very light limited environment, and one would anticipate that their eyes would be extremely sensitive, and therefore demonstrate relatively low temporal resolution. Previous studies have determined this to be the case for other species, and this again proved to be true for the species in this study. However, the exceptions to this rule are the euphausiid crustaceans, particularly those possessing bilobed eyes. The bilobed crustaceans have a modified 2$^{nd}$ and/or 3$^{rd}$ pleopod, which is extremely long and frequently has the claws at the end, and it has been suggested that this is an adaptation for a predatory lifestyle. If these species are indeed actively tracking prey, one might anticipate that they would need a higher temporal resolution, and previous studies have determined that this is indeed the case – the bilobed crustaceans have extremely high temporal resolution, on par with that of their shallow water relatives. The optics of the upper lobe differ from the optics of the lower lobe, with the upper lobe

being adapted for lower sensitivity, higher visual acuity, and the lower lobe being adapted for higher sensitivity, lower visual acuity. Based on this, one might anticipate that the temporal properties of the receptors cells of the upper lobe would also be different from those of the lower lobe. I was able to conduct 6 experiments on three species of bilobed crustaceans in which I obtained good recordings from both the upper and lower lobes, and the upper lobe clearly has higher temporal resolution that the lower lobe. In addition, euphausiids also prove to be different from all the other species I have studied to far, in that there is a clear change in temporal resolution with light adaptation. In 20 of the experiments I conducted on a variety of species, I measured the temporal resolution of both a fully dark-adapted eye, as well as one that had been light adapted with a dim light for 1 hour. All species showed no change in temporal resolution with light adaptation, with the exception of the euphausiids. Both bilobed and monolobe species showed significant enhancement of temporal resolution due to light adaptation. This brings up the very real possibility that these species might also demonstrate a circadian rhythm in sensitivity, which I will test the next time I have access to some midwater euphausiids.

In addition to collecting data for my own research, I was also able to obtain some samples from the Oxfam net for a colleague at Illinois Wesleyan, who is interested in determining if larval ophiuroids make it to waters this far south.

In spite of the early winch problems, this has one of the most productive cruises I've ever been on, due to the combined efforts of the crew and technicians to get us the animals we needed, the wonderful achievements of the catering staff, and the facilities available on the *RRS Discovery*.

**Tammy Frank**

### 9.3.4 Fishes

**Midwater collections** - The current collections confirmed the reputation this sampling area has for its richness in the diversity of meso- and bathypelagic fish species. A noteworthy exception, however, was the relative paucity of myctophids, especially large ones, in the catches from all levels. The number of ceratioid anglerfish collected was substantial, with over 100 *Melanocetus*. An unusually large (183 mm standard length (SL)) *Caulophryne pelagica* was captured in perfect condition, due perhaps to a lethargy induced by a prodigious meal which had expanded the stomach in excess of the standard length. Among the other interesting anglers was a specimen of an unsual species of spiny *Himantolophus*, which might prove to be undescribed. Another interesting group well represented were the opisthoproctid fishes. While some *Opisthoproctus* were taken, the most common member of this family was a species of *Dolichopteryx*, a normally little-caught genus, together with several specimens of the rarer *Bathylychnops* and one *Rhynchohyalus natalensis*. Perhaps most remarkable of all was the capture of a 1230mm SL *Alepisaurus ferox* (Alepisauridae), a potentially very active species which would be expected to avoid the 8 sq. m. opening of the RMT.

**OTSB collections** - Some 16 OTSB hauls have been taken previously in the general area ($14^0$--$21^0$ N and $17^0$--$21^0$ W). Of these four were made on the slope (885--1472 m), 10 on the rise (2160--3939 m) and two in the abyss ( >4000 m). Thus the three hauls made on this cruise added information to the last two categories.
Overall 130 fish were caught, which represented 19 species and seven families (Synaphobranchidae - 3 spp., Halosauridae - 1, Alepocephalidae - 3, Ipnopidae - 3, Bathysauridae - 1, Macrouridae - 4, Ophidiidae - 4). These broadly represent the assemblage composition expected from former catches in these soundings in the region. Four species were common to all catches (*Histiobranchus bathybius, Rinoctes nasutus, Coryphaenoides (Lionurus) carapinus* and *C. (Nematonurus) armatus*), with *Bellocia koefoedi* and *Bathypterois grallator*, in addition, common to the catches on the rise. Most fish were caught in the abyssal catch (66, 11spp.), with fewer individuals but similar species richness sampled on the rise (21, 10 spp. and 43, 10 spp.).

**Samples collected** - A limited number of specimens were preserved for the fish collection at The Natural History Museum, London. The unusual opisthoproctid fishes *Dolichopteryx* (some ten specimens) and *Bathylychnops* (three) were taken, together with the one specimen of *Rhynchohyalus natalensis* captured. The magnificent specimen of the large female ceratioid angler, *Caulophryne pelagica*, was also preserved. Numerically, however, the main part of the collection was comprised of early life history stages of a wide variety of pelagic and demersal fishes needing further identification.

Tissue samples from white muscle, heart, liver gonad and kidney were taken from 12 species of midwater and demersal fishes, also for the NHM. These were deep frozen for the Museum's DNA data bank.

Blood smears were made for Dr Angela Russell (Kingston University) to continue joint work on blood parasites and cell morphology. A total of 28 slides were made from 13 species of midwater and bottom-living fishes.

A Percy Sladen Memorial Fund grant was obtained for my travel and other expenses associated with the cruise and I am indebted to the Trustees of the Fund for this award.

**Nigel Merrett**

### 9.3.5 Phylogeny of Deep Sea Fishes

The main objective was to collect specimens of the largest possible number of species of fish inhabiting the deep sea. For large individuals, rather than keep the all the body, it was sufficient to retain portions of the white muscle and liver. All the samples were preserved in 95% ethanol, which is not the ideal fixative for keeping organisms as collection but ideal for further studies, especially for extraction of the DNA. The aim of my work, once I am back at the University of Bristol, is to sequence of the most suitable genes from the mitochondrial genome for a phylogenetic study. The results are expected to give a clearer view of the evolution of these species.

My collection of fish is composed of 60 specimens belonging to 30 different species grouped into 20 families. In addition, I have preserved in formalin several organisms including sponges, shrimps, squids, ctenophores, jellyfish, crustaceans, barnacles and fishes to enlarge the collection of material used for undergraduate teaching in Zoology at the University of Bristol.

Acknowledgements
I am very grateful to Dr. J.C. Partridge for inviting me to take part to this project. Thanks to the crew and the scientific party that allowed me to spend a memorable time during this six weeks cruise.

**Sergio Stefanni**

### 9.3.6 Bioluminescence and Physiology

**Objectives**

1. Collect data on the red and blue-emitting photophores of the fishes *Malacosteus, Pachystomias* and *Aristostomias.*
2. Examine the size and distribution of ventral photophores in gonostomatid and other fishes in relation to the ventrally-projected area of the body.
3. Collect decapod gill material for Southampton Ph.D. project
4. Investigate anglerfish bioluminescence and its control (with **Spencer Nyholm**; Section 9.3.7)
5. Determine the distribution of coelenterazine in deep-sea species (with **Jean-Francois Rees**; Section 9.3.8)
6. Acquire data on the ambient bioluminescence at the sea floor using the ISIT lander (with the **Aberdeen group**; Section 9.1)
7. Obtain material for subsequent morphological study of particular decapod photophores and eyes (with **Peter Shelton**; Section 9.3.1)

Objectives 4-7 will be included in separate reports by the named collaborator

**Achievements**

1. Six *Aristostomias*, seven *Pachystomias* and >20 *Malacosteus* were taken during the cruise. The orbital photophores were examined and their fluorescent characteristics determined. The emission of red light by these species is correlated with the presence of large amounts of red fluors in the photophores, and it is very likely that their bioluminescent emission spectra are some function of these fluors.

The data show that there are fundamental differences between the species in their fluors and their distributions. The known emission maximum of *Malacosteus* (maximum at 708nm) is entirely explicable as derived from the emission of the fluor in combination with the transmission of the overlying brown filter. Bioluminescent

spectra of *Aristostomias* and *Pachystomias* have not yet been determined (one of the objectives, with Edie Widder, that could not be achieved). Their fluors suggest a shorter wavelength bioluminescence emission, and neither species has a filter pigment in the aperture. The pre-orbital photophore of *Pachystomias* has a spectrally different fluor to the suborbital, suggesting that their bioluminescence emission maxima may also differ.

Photophore material has been fixed for structural study, and frozen for identification of the fluor (Prof. W. Rudiger, Univ. of Munich).

The bioluminescence emission of both red- and blue-emitting photophores of some specimens of *Malacosteus* and *Pachystomias* was successfully recorded (with Edie Widder) using an image-intensified videocamera.

2. Some photographs of the ventral surfaces of a number of gonostomatid and myctophid fishes were taken early in the cruise with the intention of assessing the relative areal cover and individual separation of the photophores by later image-processing. The time required to deal with the desired number of specimens was incompatible with other activities; instead specimens were preserved separately for later processing ashore. These included sternoptychids, gonostomatids, photichthyids and myctophids as well as some enoploteuthid squids.

3. Gills from a range of oplophorid decapods were preserved in Karnovsky's fixative for anlaysis of their structure and surface area in relation to the depth distribution and lifestyle of the different species (Hannah Dutton, SOC Ph.D. student).

In addition to these activities a variety of fish and squid material was preserved for later morphological studies. Three species of decapod crustaceans were preserved in alcohol for later DNA studies (Torin Morgan, SOC). A number of RMT1 samples were divided in two and the two halves preserved in formalin and in ethanol for assessment of the practicability of using formalin-preserved sediment trap material for DNA determination of the main components (Alex Rogers/Richard Lampitt, SOC).

**Peter Herring**

### 9.3.7 Light organs of deep-sea anglerfishes

This report describes work to undertaken in association with **Peter Herring.**

Some luminescent fishes and squids derive their light from bacteria symbiotic within light-emitting organs. In nine of the eleven families of deep-sea Ceratioid anglerfishes, females house luminous bacteria in their light organs (escas), the light of which is believed to be used to attract prey. In the family Ceratiidae, females have additional sessile glands (caruncles), which house luminous bacteria and are located anterior to the dorsal fin. Our goal with the samples collected during D243 is to compare certain aspects of the escas and caruncles of deep sea anglerfishes to the light organ of the well-studied shallow water Sepiolid squid *Euprymna scolopes*, used as a model organism for the study of the effects of mutualistic bacteria on animal host tissue. Like *E. scolopes*, anglerfishes are believed to house a monoculture of *Vibrio* sp. bacteria as extracellular symbionts. How bioluminescent animals establish, maintain, and regulate high-density monocultures of bacteria in a organ which is open to the surrounding bacteria-rich sea-water is a process which is not thoroughly understood and is the focus of several laboratories studying the *Euprymna/Vibrio* model. The symbiotic escal light organs and caruncles obtained from the angler fish of D243 were prepared for analysis as follows:

1. To compare the morphology and ultrastructure of the epithelial tissue which surrounds the symbionts in the escas and carucles of anglerfishes to the light organ of *E. scolopes*, escas and caruncles have been fixed and embedded in plastic resins. The samples will be sectioned and viewed by light and electron microscopy. Some characters of the *Euprymna* light organ tissue which we will use for comparison are epithelial cell shedding and the presence of phagocytic macrophages, which are thought to play a role in the regulation of the *Euprymna/Vibrio* association.
2. Escas and caruncles were also fixed and prepared for immunocytochemistry. In the squid light organ, the presence of a peroxidase related to human myeloperoxidase (MPO) has been described. MPO is involved in the production of potent antimicrobial hypohalous acids. Using antibodies to MPO, we will look for the presence of this enzyme in anglerfish escas and caruncles.
3. The tissues from escas and caruncles were frozen and will be used for the following:
   a. Frozen tissue will be used in a spectrophotomeric assay that can detect peroxidase activity.
   b. The proteins of the squid light organ have been studied using two-dimensional poly-acrylamide gel electrophoresis (2D-PAGE) which separates proteins based on molecular weight and charge characteristics. The proteins of escas and caruncles will be analyzed by 2D-PAGE and compared to those found in the squid light organ.
   c.. The exact identity of the symbiotic partners in the escal light organs remains unknown.These bacteria have not yet been cultured although many attempts have been made. Previous studies have shown that the symbionts of two species of anglerfishes (*Melanocetus johnsoni* and *Cryptosaras couesi*) belong to the genus *Vibrio* but are not one of the four typical species associated with light organ symbioses. Bacterial DNA from the frozen symbiotic tissues will be isolated and purified. Universal primers to 16S ribosomal RNA will be used to amplify bacterial DNA. These PCR amplified products will be cloned and sequenced in an attempt to identify the symbionts.
4. Symbiotic tissue was also placed in an RNA preservative and frozen. In this state it will be possible to isolate mRNA from these samples and in the future comprise cDNA libraries of anglerfish escas and caruncles.

In total, 107 anglerfish were caught during D243. There were representatives from seven families listed below:

| Family | | Number caught |
|---|---|---|
| **Ceratiidae** | | |
| | *Cryptosaras couesi* | 13 |
| | *Ceratias holboeli* | 6 |
| **Chaulophrynidae** | | |
| | *Chaulophryne pelagica* | 1 |
| **Diceratiidae** | | |
| | *Diceratias sp.* | 1 |
| **Himantolophidae** | | |
| | *Himantolophus groenlandicus* | 1 |
| | *Himantolophus sp.* | 2 |
| **Linophrynidae** | | |
| | *Linophryne coronata* | 1 |
| | *Linophryne madarensis* | 1 |
| **Melanocetidae** | | |
| | *Melanocetus johnsoni* | 61 |
| **Oneirodidae** | | |
| | *Chaenophryne draco* | 1 |
| | *Chaenophryne longiceps* | 3 |
| | *Dolopichthys danae* | 1 |
| | *Dolopichthys sp.* | 1 |
| | *Leptacanthichthys sp.* | 1 |
| | *Oneirodes carlsbergi* | 1 |
| | *Oneirodes sp.* | 5 |
| | Unidentified Oneirodids | 8 |
| | **Total** | **107** |

In addition, the following fishes or squids, which also have bacterial light organs, were prepared for analyses as described above:

| Species | Number of samples prepared |
|---|---|
| *Opisthoproctus soleatus* | 7 |
| *Winteria telescopa* | 1 |
| *Rhynchohyalus sp.* | 1 |
| *Heteroteuthis sp.* | 1 |

Overall, the numbers and species of fish containing bacterial light organs should be more than adequate to complete the goals of this study.

**Funding and costs**

A grant covering travel and partial supplies for D243 in the amount of $2,000 (U.S.) was awarded to SVN by the Honolulu chapter of the Awards in Research for College Scientists (ARCS) Foundation. Other supplies and shipping costs (approximately $500, U.S.) were donated by M. McFall-Ngai at the University of Hawaii and PJH at SOC. The estimated costs of future supplies and fees paid for electron microscopy facility usage to complete this study amounts to approximately $1,000-$2,000 (U.S.).

**Acknowledgements**

SVN would like to thank Peter Herring and Julian Partridge for the invitation and opportunity to participate in D243. I would also like to thank the funding sources listed above.

**Spencer Nyholm**

### 9.3.8 Biochemistry of Bioluminescence

**Scientific objectives:**

My work focuses on the evolutionary relationships that could exist between bioluminescence, the emission of light by living organisms, and antioxidative defence mechanisms. These mechanisms are essential to all life forms as they prevent oxidation of cellular constituents subjected to oxygen and its reactive species, such as hydrogen peroxide. Our previous works suggest that marine bioluminescence mechanisms could have been derived from enzymatic and non-enzymatic defence mechanisms dealing with the toxicity of oxygen. Coelenterazine is a small molecule acting as luciferin (light-emitting substrate) in most marine bioluminescent organisms, from protozoa's to fishes. Coelenterazine has also recently been shown to be a remarkable antioxidant. Our model suggests that coelenterazine first evolved as an antioxidant in surface waters where oxidative dangers are high. It shifted toward its present light-emitting role when animals colonised deeper layers of the oceans where problems posed by oxygen toxicity should be markedly reduced. Accordingly, defence mechanisms against oxygen toxicity should be lower in deeper living animals.

Despite its wide occurrence in marine bioluminescence, the sources of the luciferin remain to be identified. Some previous work suggests that *de novo* biosynthesis of coelenterazine could take place during the development of eggs from the shrimp *Systelapsis debilis*. However, doubts persist and more work based on improved detection methods is required.

**Our objectives on this cruise were:**

1. To analyse the possible adjustment of metabolism, antioxidative defence mechanisms (enzymatic and non-enzymatic) and coelenterazine content in pelagic fish to the reduced oxidative threat in the deep-sea.
2. To compare the antioxidative defence arsenal of benthic fish to that of the pelagic forms.
3. To investigate the occurrence of oxidative damages in cells of deep-sea fishes brought to the surface.
4. To analyse the possible protective effects of coelenterazine on deep-sea fish red blood cells subjected to oxidative stress *in vitro.*
5. To further analyse the possible *de novo* synthesis of coelenterazine in the eggs of the shrimp *Systellapsis debilis*.

Tissues (liver, white muscle, skin) from 848 individuals of 75 fish species were collected and frozen. Blood was collected and centrifuged on-board as to obtain plasma. Some tissues were homogenised and chemiluminescence-based biochemical assays were performed on the ship ; others will be carried out once in Belgium. These include HPLC-ECD assays for coelenterazine, antioxidative vitamins

(vitamin C and E), glutathion. Chemiluminescence-based assay methods for enzymes such as superoxide dismutase, catalase, glutathion peroxidase will also be applied to all samples. The activity of citrate synthase, an enzyme involved in the energy-supply of cells, will be used as an index of metabolic activity. Also, the total reducing power of the plasma will be analysed. Evidences of oxidative damages to cellular DNA in the blood cells and tissues of fishes brought to the surface will also be searched for by Single Cell Gel Electrophoresis and HPLC-ECD.
Last but not least, the role of pollutants such as polychlorinated compounds in the induction of antioxidative defence mechanisms will also be investigated on fish livers.

On-board experiments were carried out on red blood cells isolated from different species subjected to hydrogen peroxide. Also, the ability of coelenterazine to protect red blood cells from surface and deep-sea fishes was investigated.

*Systellapsis debilis* eggs and females (85 individuals) have been collected. The developmental stage of the eggs was determined. Assays of coelenterazine, oxidation products and derivatives will show whether a net synthesis of coelenterazine occurs in the eggs. Also, we will investigate the possible maternal transfer of the luminescence substrate from the mother to the unfertilised egg.

This work should help us better understand the evolutionary roots of marine bioluminescence.

**Acknowledgements**

My participation to this cruise has been supported by a grant from *the Fonds National pour la Recherche Scientifique (FNRS),* Belgium (£900). Other grants obtained from various sources will pay for the assays (£4000).

**Comments**

I have very much appreciated the kindness and professionalism of the officers and crew of *Discovery*. Also, an excellent collaboration between scientists allowed us fully exploiting most of fish collected. This was an excellent cruise in terms of quality and quantity of the material collected.

**Jean-Francois Rees**

### 9.3.9 Anatomical Studies of Deep Sea Fishes

**Aims:**

1. Comparative morphology of deep sea fish brains with special emphasis on sensory input
2. Comparative morphology of pineals in deep sea fish brains
3. Melatonin content of deep sea fish brains and retinae
4. Analysis of retinal ganglion cell morphology

**Statistics:**

No. of specimens collected: 210, more than 50 midwater species; 29 demersal species
No. of brain dissected and stained: 70
No. of retinae cultured and backlabelled with dextranes for RGC analysis: 23
No. of brains & retinae cryofixed for melatonin assay: 26

The diversity, size spectrum and quality of the fish caught was excellent and provide a sound basis for further evaluation in Tuebingen.

## Comparative morphology of deep sea fish brains with special emphasis on sensory input

The central nervous system of teleosts has been shown to vary greatly in overall morphology with those parts particularly large, which in behavioural experiments had been found to provide input of special importance. Well studied examples are electric fish with an enormous cerebellum, catfish, with reduced optic tectum and large (gustatory) vagal lobes, and goldfish or trout with large optic tecta. In the case of most deep sea fishes, very little is known about the behaviour and about the senses which are most used for predation, predator avoidance, or mating. We therefore propose an approach which is the reverse of the above observations: We want to study the specialisations of their brains and the differentiation of the afferent cranial nerves in order to make predictions about the sensory modalities most used by a given species in the adaptation to its particular way of living.
For this purpose, brains were fixed in paraformaldehyde and dissected so that the brain and cranial nerves were exposed in a quasi mid-sagittal section. The topography and origin of the nerves was also determined, as well as special differentiations of the sensory organs. Subsequently, two lipophilic dyes of different colour (DiI: red, DiD: blue) were applied to the nerves; these travel by diffusion along the plasma membranes of the axons and, in the end will delineate the cerebral areas dealing with processing of the information carried by a particular nerve. In several brains, up to 12 different nerves or major branches could be stained in this way. Cursory preliminary observations indicate major differences in the diameter of the individual nerves and the size of related brain areas. Examples are: Anglerfish and gulper-eels with tiny eyes/optic nerves and well developed trigeminal nerves; another example of an extremely large trigeminal nerve is the *Bathytyphlops*. Large noses and associated olfactory nerves and bulbs were observed in *Haptenechelys texis* and *Iliophys brunneus.* Large eyes / optic nerves and tecta are present in myctophids, and some hatchetfishes (*Sternoptyx*).

Confirmation of these findings will be based on sectioning the brains in order to identify and determine the extent of the projections areas and the count of the number of axons carried in each of the sensory nerves. For the latter purpose, brains and nerves were also fixed in glutaraldehyde. The evaluation of this material will be done in collaboration with Dr. S.P. Collin, Univ. of Brisbane, and Prof. A. Popper, Univ. of Maryland.

In addition to providing circumstantial evidence for the sensory specialisations of a many midwater and demersal teleosts these investigations will also serve as a reference and basis for studies of localisation of particular physiological makers such as melatonin binding sites or the expression of melatonin receptors (Priede et al., in press).

## Comparative morphology of pineals in deep sea fish brains

The pineal glands of the diencephalon are a major source of melatonin production in the vertebrate brain; their main function is thought to control the seasonality of reproductive behaviour. In most teleosts, pineals contain functional photoreceptors, the visual pigment absorption spectra and molecular biology is studied by Profs. Bowmaker and Hunt, also on this cruise.

During the course of the brain dissections (see 1) it became evident that there is a considerable variation in the size and location of pineal glands, particularly in midwater fishes. Most often, they were located beneath a clear, transparent spot in the skull and skin, making it likely, that they are capable of catching photons. In some cases of thick skulls, there was a special canal allowing the positioning of the pineal almost directly underneath the skin. Electron microscopic studies will show, whether functional photoreceptors are present and whether these show adaptations to the low light environment similar to those found in the retina.
Whilst in all of the over 50 species of midwater fish examined, pineals could be identified without fail, the situation in the demersal species is less clear. Due to the large cavity formed by the skull, serving to accommodate large semicircular canals, there is a considerable distance between ther brain itself and the roof of the skull, where the pineal gland is expected to be localised. The space is filled with a gelatinous substance and stabilised by numerous meningeal trabeculae, making the identification of a pineal stalk or the pineal itself difficult. In two cases, however, structures have been identified on the roof of the skull which appear to be good candidates for pineals (pending confirmation by ultrastructural studies). Careful dissection of additional fixed material will show whether pineals are present as a rule in demersal fish, too.
Even though they would not be expected to perceive light – no “window” in the skull was observed in the demersal fish - these pineals could still function as melatonin production sites similar to the situation in mammals, where photosensitivity has also been lost, and the rhythmic activity controlled by the suprachiasmatic nucleus.

## Melatonin content of deep sea fish brains and retinae

The presence of pineals on the one hand and of both functional melatonin binding sites, and melatonin receptor expression in specific cell populations of a number of demersal gadiform fish (Priede et al. in press), on the other make it necessary to provide direct evidence for the presence of melatonin in the brains of deep sea fishes. Therefore, native brains were quickly frozen and kept at –80°C. Upon transferral of the material to Tuebingen, the melatonin content will be determined by HPLC and electrochemical detection.
Since retinal photoreceptors are also capable of producing melatonin (and the ocular size and volume of rods remarkably large in several of the demersal and midwater fishes) isolated retinae were also collected in the same specimens for determination of melatonin content. The results will potentially indicate whether the retinae may have substituted the pineals as the major source of melatonin for a particular species.

## Analysis of retinal ganglion cell morphology

Studies of the topography of retinal ganglion cells by S.P. Collin have indicated a high degree of specialisation in many of the midwater and demersal fish eyes. In mammal retinae, functional specialisation of the visual system is reflected by a set of different ganglion cell classes in the retina which are clearly defined on the physiological and morphological level. It is therefore interesting to determine to what extent, such a differentiation can also be found in the retinae of deep sea fishes. Ganglion cells were labelled be application of fluorescent dextrane molecules to the optic nerve stump, and by keeping eyes in a culture system for two days. This interval is sufficient to allow the trace to be transported by axoplasmic transport to the perikaryon and even into the proximal dendrites, allowing characterisation and identification by means of a laser scan microscope.

## Collaborative activities:

Prof. Priede: brain morphology, melatonin content; pineals in demersal fish
Pros Bowmaker and Hunt: morphology of pineal photoreceptors in deep sea fishes
Dr. S.C. Collin: axon counts in sensory cranial nerves

Prof. A. Popper: morphology of inner ears in deep sea fishes

**Jochen Wagner**

### 9.3.10 The pineal organ of deep-sea fish

This is joint project with **Professor Hans-Joachin Wagner**, Anatomy Institute, Tübingen University, Germany and **Professor David Hunt**, Department of Molecular Genetics, Institute of Ophthalmology, University College London.

A NERC small project grant to cover the travel costs and consumables for D243 was awarded to JKB. A full project proposal is in preparation (JKB and DMH) for studies of teleost pineal photoreceptors and photosensitive pigments which will include work on the deep-sea pineal organs collected in D243. Funding for postdoctoral support to contribute to the microspectrophotometry and the molecular genetics will be requested.

**Objectives**

We plan to examine the organisation of the photoreceptors and the structure of the photosensitive (non-visual) pigments of the pineal organs of representatives of a number of teleost groups that inhabit widely different photic environments, from shallow freshwater to the deep ocean. Applying microspectrophotometric techniques, we aim to establish the number of spectrally different photoreceptors, their spectral properties and their relative proportions. In parallel with the physiological study, we plan to examine, both by light and electron microscopy, the detailed morphology of the photoreceptors and their arrangement within the pineal vacuole. A third parallel study will focus on identifying and sequencing the genes coding for the photosensitive pigments of the pineal and to use this information to determine the amino acid structure of the opsins. We plan to express the opsins *in vitro* and by comparing the amino acid sequences and the spectral sensitivity of the pigments we shall identify potential spectral tuning sites for pineal opsins.

Deep-sea fish offer a singular opportunity for extending our understanding of pineal photo-pigments including their spectral absorption, opsin structure and evolution. The extensive knowledge of deep-sea ocular visual pigments from a wide range of species will allow correlations to be made between these pigments, normally rod-based rhodopsins, and the pineal pigments. Comparisons of the amino acid sequences of retinal and pineal opsins may determine regions of opsin that contribute to the slower response kinetics of pineal photoreceptors.

**Work on board**

Pineal organs and liver tissue were collected from a range of mid water species trawled from between 1200 and 300 m. These include hatchet fish (Sternoptychidae), *Sternoptyx diaphana, S. pseudobscura, Argyropelecus affinis, A. gigas* and *Polyipnus polli*, the viper fish (Chauliodontidae) *Chauliodus sloani* and the light fish (Gonostomatidae) *Gonostoma longata*. These species were selected because they can be collected in relatively large numbers and some show diurnal migrations (especially *Chauliodus*) moving towards shallower depths during the night.

*Microspectrophotometry.* Pineal organs were removed from ‘dark adapted’ fish collected either at night or in the closed cod end during the day. Pineals were dissected under dim red and lightly fixed in 2% glutaraldehyde/paraformaldehyde for 15-30 sec. The tissue was then stored in saline containing antibiotic and antimycotic agents at 4ºC, a procedure that has been routinely used to preserve retinal visual pigments for up to six months. This tissue will be taken back to the Institute of Ophthalmology for microspectrophotometric analysis (JKB).

| **Species** | **Number of pineals** |
|---|---|
| *Chauliodus sloani* | 16 |
| *Sternoptyx pseudobscura* | 15 |
| *Sternoptyx diaphana* | 20 |
| *Argyropelecus gigas* | 5 |
| *Argyropelacus affinis* | 19 |
| *Polyipnus polli* | 2 |
| *Gonostoma longata* | 3 |

Microspectrophotometry is a technique that enables the measurement of photosensitive pigments within the outer segments of pineal photoreceptors. Our instrument is a modified dual-beam Liebman microspectrophotometer with a cooled photomultiplier. For recording pigment spectra, a small piece of tissue (about 2 mm sq.) or the entire pineal is teased apart on a microscope slide and outer segments probed with a measuring light beam normally about 2 μm square, but which can be adjusted to fit within the dimensions of the cell. Spectra are routinely recorded from 750 to 350 nm. We have used the instrument in extensive studies of teleost, avian and primate retinas.

*Anatomy.*
Isolated pineals and whole brains from all species were fully fixed in buffered glutaraldehyde/ paraformaldehyde for histological examination by both light and electron microscopy. This work will be undertaken in Tübingen (H-JW).

*Cloning and sequencing of pineal opsin cDNAs.*
Substantial numbers of pineals were removed in the light and placed into '*RNA Later*'. Liver samples were also collected for isolation of genomic DNA.

| Species | Number of pineals |
|---|---|
| *Chauliodus sloani* | 63 |
| *Sternoptyx pseudobscura* | 20 |
| *Sternoptyx diaphana* | 34 |
| *Argyropelacus affinis* | 36 |
| *Polyipnus polli* | 10 |

We will use a cDNA approach to the cloning and sequencing of the pineal opsin genes. By doing so, we can be confident that the genes are expressed in the pineal. PolyA$^+$ mRNA will be isolated for RT-PCR amplification of opsin cDNAs from frozen pineal tissue using standard protocols. Primers will be designed to conserved regions of the different classes of vertebrate opsin genes (initially rod opsins) and used for the amplification of fragments of the transcribed sequence. Full length copies of the cDNAs will then be generated by 3' and 5' RACE techniques. We now have considerable experience of using such a cross-species PCR approach to isolating opsin gene sequences and in extending sequence by 3' and 5' RACE.

It is possible that the opsin sequences may be difficult to isolate in this way. If so, we shall generate a pineal cDNA library. This library will be screened with opsin probes in order to identify any additional opsin genes that are expressed in this tissue. This library will also provide a valuable resource for the identification of genes that underlie other components of phototransduction in the pineal. This work will be carried at the Institute of Ophthalmology (DMH).

Project background: Non-mammalian vertebrates possess photoreceptors in their pineal complex and deep brain as well as the retina. The extraretinal photoreceptors are believed to be essential for seasonal reproduction and regulation of circadian physiology. Even deep-sea organisms living in extremely low (or absence) solar light

levels show seasonal behaviour. Pineal photoreceptors probably function primarily as luminance detectors: the absence of any focussing mechanism and the irregular organisation and convoluted nature of the pineal epithelium suggests that only diffuse light reaches the pineal. In addition, the high convergence of photoreceptors to neurons and the slow time course of pineal photoreceptor responses imply that the pineal cannot distinguish discrete, rapidly changing light stimuli. The pineal is thus designed to detect slowly changing ambient light levels and possible their spectral composition.

Although pineal photoreceptor cells are similar to those of the retina, their photopigment content remains poorly investigated. Most studies of spectral sensitivity have been conducted in salmonids and imply photopigments with wavelengths of maximum absorbance ($\lambda_{max}$ ) at about 495 nm and 520-530 nm. In contrast, microspectrophotometry (msp) suggested photoreceptors with $\lambda_{max}$ at about 463 nm and 561 nm. Intracellular recordings from pineal photoreceptors in a cyprinid, the goldfish, also gave a $\lambda_{max}$ around 530 nm. The consensus is that fish pineal photoreceptors are dominated by pigments with $\lambda_{max}$ around 520-530 nm which is close to the $\lambda_{max}$ of many teleost retinal rods and middle-wave-sensitive (MWS) cones, but that a second, spectrally distinct longer-wave receptor may be present.

Are these photopigments identical to retinal visual pigments? Are they based on retinal opsins or are the pigments specific to the pineal? Studies using immunocyochemical labelling of pineal photoreceptors, using a range of antibodies (e.g. COS and OS) raised against retinal opsins indicate that both rod-like and cone-like opsins may be present. More recently a number of novel opsins have been identified in vertebrates that do not belong to either the retinal rod or four retinal cone opsin families. At least two of these, VA opsin and parapinopsin have been located in areas associated with the teleost pineal.

Preliminary data: We have microspectrophotometric data from isolated intact pineal photoreceptors from shallow-living freshwater cyprinids (goldfish, *Carassius auratus*; orfe, *Leuciscus idus*) and a characid, the cavefish, *Astyanax fasciatus*. Analysis of outer segments from the pineal of goldfish revealed a single photopigment of $\lambda_{max}$ 512 nm. This value is different from any of the retinal photoreceptors, but is most similar to the rod (523 nm) and MWS cone (530 nm). Preliminary analysis of pineal cDNA has identified a rod-like gene expressed in the pineal. Partial sequencing shows about 84% homology with the retinal rod opsin.

**Jim Bowmaker**

### 9.3.11 Cephalopod visual pigments

The visual pigment rhodopsin is central to the visual system of vertebrates and invertebrates. We now have a basic understanding of the mechanism whereby the spectral sensitivity of vertebrate pigments is achieved, but this process in invertebrates is far less well understood. The cephalopods with their well-developed eyes provide one of the best natural systems in which to study this. The main aim of this work is therefore to extend our knowledge of cephalopod rhodopsins in terms of spectral sensitivity and evolution.

**Background**

The Cephalopod molluscs are divided into two sub-classes, the Nautiloidea, comprising a single Order Nautilida, and the Coleoidea, comprising four Orders, the Sepiida, Teuthida, Vampyromorpha and Octopoda. The rhodopsin gene is one of the few genes that has been looked at in any detail in the Cephalopods: the gene has been sequenced in a single species of octopus (Ovchinnikov *et al*., 1988), in two

Myopsid (Hall *et al*., 1991; Morris *et al*., 1993) and one Oegopsid squid (Hara-Nishimura *et al*., 1993), and in a single species of cuttlefish, *Sepia officinalis* (Bellingham *et al.*, 1998). Our contribution has been the sequences of one of the Myopsid squids, *Alloteuthis subulata* (Morris *et al*., 1993), and the cuttlefish, *S. officinalis* (Bellingham *et al.*, 1998).

Our interest in Cephalopod rhodopsin arises from the observation that different species occupy different depth habitats and show correlations between increasing depth and shortwave spectral shifts of their visual pigments. In this regard, they show parallels to species of fish that occupy deep-water habitats and display shortwave shifts in the $\lambda_{max}$ of their visual pigments at increasing depths (Partridge *et al*., 1989). We have now completed two detailed studies of fish visual pigments and have been able to identify candidate amino acids responsible for these spectral adaptations (Bowmaker *et al*., 1994; Hunt *et al*., 1996; Hope *et al*., 1997; Dulai *et al*., 1999). Similarly, by comparing the deduced amino acid sequences in those cephalopod species where both the gene sequence and the $\lambda_{max}$ of the rhodopsin pigment have been obtained, we have made some progress in identifying tuning sites in these pigments (Bellingham *et al*., 1998); in many cases, these turn out to be similar to the substitutions identified in vertebrates. However, the number of species in which gene sequence and $\lambda_{max}$ data are available is limited, so any model can be at best speculative. The study of additional species will serve to confirm or modify this model and to establish the extent of convergent evolution in vertebrate and invertebrate opsins in response to a common selective pressure of visual adaptation.

## Methodology

Eye tissue will be used for visual pigment spectroscopy and for the isolation of retinal mRNA. Other soft tissue will be used for the isolation of genomic DNA. Pigments will be purified by protocol described in Morris *et al*. (1993). PolyA+ RNA and will be isolated by standard methods. The rhopdopsin gene sequences will be polymerase chain reaction (PCR) amplified from both genomic and cDNA using primers designed to known conserved regions of the gene. We have already had considerable success with these methods in cephalopods and in a range of other vertebrate and invertebrate species. The amplified fragments will be cycle-sequenced on an “in-house” ABI 373a automated sequencer. Phylogenetic relationships between gene sequences will be examined using a range of methods including neighbour-joining and parsimony. We will also investigate the possibility of expressing recombinant opsins and regenerating these *in vitro*.

## Collection of tissue samples on board ship

During the course of *RRS Discovery* cruise D243, tissue samples from the following have been collected:

*Vampyroteuthis infernalis*, the single member of the Vampyromorpha, six examples of Oegopsid squid, *Histioteuthis*, *Mastigoteuthis*, *Chiroteuthis*, *Sthenoteuthis*, *Bathyteuthis*, and *Octopo-teuthis*, and two species of octopus, *Vitreledonella richardi* and *Alloposus mollis*.

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**David Hunt**

### 9.3.12 Molecular adaptations to high pressure and low temperature in deep-sea fish

This is joint project with **Professor Martin J Warren**, School of Biological Sciences, Queen Mary and Westfield College, London and **Dr Julian C Partridge**, School of Biology, University of Bristol, Bristol.

A full project grant proposal has been submitted to BBSRC for funds for this project.

**Background**

The physical properties of the deep-sea create a unique environment, which is characterised by high pressures and low temperatures. Surprisingly, very little is known about the molecular changes that enable the proteins of deep-sea fish to function at high pressure and low temperature, although it is clear that molecular adaptations have taken place, leading to alterations in the kinetic and thermal properties of proteins (Swezey and Somero, 1982; Siebenaller, 1984; Somero, 1992a).

The pressure of up to 120 Mpa in the deepest areas of the oceans is greater than the pressures known to cause single-chain proteins to undergo pressure-induced denaturation. For oligomeric proteins, dissociation occurs at much lower pressures. In one of the few studies undertaken in this research area, proteins of deep-sea fish have been shown to have an increased resistance to thermal denaturation compared to homologous proteins from shallow-water relatives. This increase in thermal stability is thought to be due to the evolution of especially rigid proteins that are able to resist disruption of tertiary and quaternary structure under high pressure ( Swezey and Somero., 1982; Somero, 1992a,b). The effect of low temperatures must be considered alongside that of pressure since the enzymes of deep-sea fish must work efficiently at $4^{o}$C.

The objective of this project is to carry out a detailed analysis of structural adaptations in the proteins of deep sea fish.

Four proteins have been selected for study, Cu, Zn-superoxide dismutase (SOD), lactate dehydrogenase (LDH), aminolaevulinic acid dehydratase (ALAD) and porphobilinogen deaminase (PBGD). SOD enzymes are a family of metalloproteins that catalyse the dismutation of the superoxide anion and are therefore part of the essential process of removing harmful cellular oxidising species. Eukaryotic CuZn SODs are 32kDa homodimers. Crystal structures of the enzyme have been solved to 2Å resolution and reveal that the active sites of the dimer work independently of each other. LDH catalyses the oxidation of NADH by pyruvate to yield lactate. The enzyme exists as a homotetramer and the structure has been solved to high resolution from a range of sources. ALAD is the second enzyme of the haem biosynthetic pathway. It is a homo-octamer and in eukaryotes other than higher plants, it appears to require one Zn ion for catalytic activity. The topological elucidation of the enzyme has revealed that the active sites within the quarternary structure act independently of each other. Moreover, the majority of the interactions that hold the enzyme complex together, hydrophobic and ionic, are mediated via the 50 or so amino acids that make up the N-terminal tail of the enzyme. PBGD is the third enzyme in the haem biosynthetic pathway. It catalyses the polymerisation of four pyrrole units (porphobilinogen) into a linear tetrapyrrole (preuroporphyrinogen). The enzyme is a monomer of $M_r$ 35,000 and is thermally very stable.

**Work on board ship**

Liver and body muscle tissue samples have been collected from the following species of deep-sea fish, *Chauliodus sloani*, *Gonostoma elongatum*, and *Borostomias elucens*, all from the Order Stomiformes, *Coryphaenoides armatus* and *C. profundicolus* from the Order Gadiformes, and *Conocara salmonea* from the Order

Osmeriformes. These have been stored at -80$^{o}$C, so it will be possible to carry out enzyme assays and to isolate mRNA for cDNA synthesis on return to the London laboratory.

## Future work

### *In vivo* assay

In each case, the enzymatic activity of each protein will be assayed using standard techniques for the determination of kinetic constants, the relationship of activity with temperature, pH dependent kinetics and thermal stability. As far as possible, these experiments will be carried out both at atmospheric and at high pressure using a pressure chamber specially constructed for this purpose.

### Amino acid sequence determination

mRNA will be isolated from liver and/or muscle samples and cDNA generated using standard methods. cDNA libraries will be set up and screened with the orthologous sequences from other species, and all positive clones will be sequenced on our in-house ABI sequencer. The deduced amino acid sequences from deep-sea and shallow-living species (and other vertebrates species) will then be compared and any candidate substitutions noted. Particular attention will be paid to the pattern of substitutions, whether a particular residue shows frequent substitution, and whether a particular region of the protein is substantially altered.

### *In vitro* analysis of recombinant proteins

The complete cDNA sequences will be used for the *in vitro* synthesis of the respective proteins. In order to maximise the production of protein, the various enzymes will be expressed as recombinant proteins in *E. coli*. and purified using standard procedures and fplc. They all have fairly simple assays allowing their purification to be monitored in terms of specific activity.

### Kinetic studies and protein characterisation

Recombinant protein will be compared to the crude tissue homogenates in order to compare Km, temperature rate profiles, pH dependency and thermal stability. This will give a good indication as to whether the recombinant enzyme has folded in a fully active form. The availability of purified protein will allow accurate rate constants to be determined at both normal and high pressure.

Finally, the large quantities of protein will provide sufficient material to permit the crystallisation of the proteins. This will allow a direct three dimensional comparison of the enzymes, enabling functional differences in enzyme properties to be identified and evaluated in structural terms.

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### 9.3.13 Visual pigments in deep-sea fish

This is joint project with **Dr Julian C Partridge**, School of Biology, University of Bristol, Bristol and **Professor Ron Douglas**, Department of Optometry, City University, London.

The samples collected during D243 will be used in two full project grant proposals to the funding agencies as follows:
(i) A study of the visual system of the stomiid dragon fishes
(ii) A study of the mechanism of spectral tuning of visual pigments and other adaptations to the spectrally- and intensity-limited light environment of the deep-sea.

**Background**

The ambient light of the deep-sea is composed of dim blue downwelling daylight and bioluminescence. However, the intensity of downwelling light diminishes rapidly with increasing depth and the limit of scotopic vision has been calculated to be at about 1000m in the clearest tropical oceans. The visual systems of deep-sea fish show numerous adaptations to this photon-limited visual environment including the loss in many species of cone photoreceptors to give a rod-only retina, and the presence of a single visual pigment in the retina with a wavelength of maximal absorbance of visual pigments at around 480 nm.

In a previous NERC-funded study of visual pigments in deep-sea fish, the rod opsin gene was sequenced in around 50 species with rod-only retinas and containing rod visual pigments with $\lambda_{max}$ values ranging from 477 to 520 nm and a number of candidate amino acid substitutions for spectral tuning were identified (Hope *et al*., 1997; Hunt *et al*., in preparation). There are however a small number of species in which more than one pigment is present (Douglas and Partridge, 1997).

Two such species are the dragon fishes, *Aristostomias tittmanni* and *Malacosteus niger*. These species appear unique amongst Stomiiformes in emitting bioluminescent light with maxima beyond 700 nm, in addition to the more usual blue light. *M. niger* possesses a pigment pair based on a single rod opsin gene (Douglas *et al.*, 1998, 1999) but utilising two chromophores, retinal and 3,4-dehydroretinal, to generate rhodopsin and porphyropsin pigments with $\lambda_{max}$ values of 517 and 550 nm respectively. This species also uses a remarkable photosensitizer based on a mixture of defarnesylated and demetallated derivatives of bacteriochlorophylls *c* and *d* in the retina to enhance the sensitivity of the "pigment pair" to its own longwave (LW) radiation (Douglas *et al.*, 1998,1999). In contrast, members of the closely-related genus *Aristostomias* lack the photosensitizer. Instead, in addition to a rhodopsin/porphyropsin "pigment pair" with $\lambda_{max}$ values of 523 and 551 nm and based on a rod opsin gene, they possess a third pigment with a $\lambda_{max}$ of 581 nm, based most probably on a second opsin protein. A number of other species also have more than one pigment based on separate opsin genes. What is unknown in all these species is whether the additional pigments are based on retained cone opsin genes or modified rod opsin genes. Cone opsins would seem best suited to the longwave sensitivity as seen in species such as *A. tittmanni*. On the other hand, the lower amplification of the cone versus the rod signal would require a cone pigment to be modified to increase its sensitivity to the low light levels of the deep sea.

**Work on board ship**

The following "multiple pigment" species have been collected:

| **Species** | **$\lambda_{max}$ of pigments (nm)** |
|---|---|

| | |
|---|---|
| | 444, 479, 505 |
| Scopelarchus analis | |
| | 464, 500 |
| Bathylagus berricoides | |
| | 474, 502 |
| Bathylagus longirostris | |
| | 484, 500 |
| Diretmus argentius | |
| | 518, 550, 581 |
| Aristostomstomias tittmani | |
| | 518, 550, 581 |
| Aristostomstomias grimaldi | |
| *Pachystomias microdon* | 515, 543 |

In all cases, eyes and liver tissue has been stoted frozen at –80ºC.

## Future work

The main objective will be to clone and sequence all opsin genes expressed in the retinas of these species. In most cases, this will be achieved by the use of PCR primer pairs designed to conserved regions of opsin genes. The fragments thus amplified will then be extended by 5'- and 3'-RACE. We will generate a retinal cDNA library for one species, *A. grimaldi*. This will enable us to clone other components of the phototransduction cascade, in particular arrestin, rhodopsin kinase, and transducin cDNAs. The spectral characteristics of cloned opsins will be confirmed by expression of recombinant opsins in mammalian cells and regeneration of the corresponding visual pigment with 11-*cis*-retinal *in vitro*. The evolutionary relationships between these sequences and their orthologues in other species will be examined by neighbour-joining and parsimony methods.

## References


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Financial support from Institute sources, £1500



**David Hunt**


### 9.3.14 Ecology of Deep-Sea Sensory Systems

**Description of Work Undertaken**

***Introduction***

My aim in all deep-sea work is to try and understand sensory adaptation to the dorsal-light dominated mesopelagic zone and the lightless depths below 1000m. Already in introducing these two subsections of the ocean, the human bias towards vision is clear as they are often described, as here, in terms of light available. It is clear from a variety of anatomical observations such as eye size, brain area for processing, nerve diameter and the relative size of other sense organs that for many deep-sea inhabitants, vision is as much a secondary sense as smell is for us. The part of these sensory systems with which I am most interested is the “front end”, that is the sensory structures themselves and my research currently centers on eyes and the lateral line vibration sense in fish.

The lateral line is one of the sensory capabilities, along with electroreception, magnetic sense and polarized light vision which humans lack all together. It enables fish to detect relatively close by water movements such as those which might be made by prey, predators moving in for the kill or possible mates. It can be thought of as an extension of the ear (indeed the hair cells which form the basic building blocks of the two senses are essentially identical) which spreads to cover the head and down the body of the fish forming the often visible line on the sides of fish after which the sense is named. In a number of deep-sea species, the lateral line has clearly become the dominant sense. The eyes may be reduced to structures akin to the simple direction finding photoreceptive cups of worms and used only for the detection of the relatively infrequent bioluminescent flashes of others. It is a fascinating task to examine the elaboration of lateral lines in the deep-sea and to try and imagine what it is like to feel ones immediate environment rather than see it.

***Work Undertaken***

This can be divided into two areas. Firstly a number of observations and measurements of visual capabilities which follow up on a surprisingly large body of work on deep-sea vision. Secondly, examination of a variety of lateral line systems of fish using description of extent and distribution to electron microscopy.

Visual capabilities of deep-sea and shallow-living fish and cephalopods

One way to assess what deep-sea fish are seeing is to look at components of the optical system such as lens and tapetum. For a variety of reasons these may be coloured. Modern technology now allows very rapid characterisation of lens transmission and transmission characteristics of the lenses of 25 species of fish and 10 species of cephalopod were measured. The aim here was to hunt for specific problems of interest and 3 of these can be identified from the preliminary results:

The most interesting problem comes not from the deep-sea but from 3 species of flying fish which landed on deck during the cruise. These 3 species, which apparently have the same lifestyle and diet possessed 3 very different lens cut-offs at 330nm, 390nm and 430nm. What, if any, is the adaptive significance of this?
All the cephalopods measured except one have high lens transmission from 320-800nm. *Vampyroteuthis* has a distinct cut-off at 410nm: Why?
Three of the 4 *Argyropelecus sp*. hatchetfish taken have yellow lenses with distinct cut-offs near 500nm but a secondary and significant window of transmission in the UV at 360nm. These three species also possess pink filters over their ventrally directed bioluminescent organs whereas the one with a lens which is transparrent over most of the spectrum, *Argyropelicus gigas*, has no pink filters. Is this of any significance? Is the large window at 360nm of adaptive significance?

## The lateral line system of deep-sea fish

This was the main research area undertaken with observations and samples taken from 35 species of fish. This includes 8 species of anglerfish, 2 whalefish and the eels *Eurypharynx*, *Saccopharynx* and *Cyema*. All these are famous for possessing long papillae or stalks on which the vibration sensitive neuromasts are mounted. Previously there was some debate on whether the sensory structure was at the base or tip of the papillum in some species and from the excellent samples taken with closing cod-end nets during this cruise, it is now clear that all are on the tip. The anglerfish samples allow completion of a project started on a previous *RRS Challenger* cruise 122 to examine and compare the proliferation of lateral line organs on these remarkable fish. Samples were fixed for scanning electron microscopy (SEM) and will be worked up on return to Australia. SEM answers questions about lateral line organ (neuromast) structure and function.

An adaptation possibly related to the presence of papillae and notable in many deep-sea fish is the possession of a gelatinous skin. It is obvious in the stomiiformes and one previously suggested function is to provide protection from the stinging tentacles of ctenophores, jellyfish and siponophores. It has become apparent that this may also function to extend lateral line organs away from the main body and thus isolate them from the fish in a manner similar to the papilla. Certainly there are many channels and inclusions in gelatinous skin demonstrating it is not just a protective layer and I have taken many samples in the hope that the gel-coat will survive for more detailed study back at the lab.

## Samples Taken for Others

*Shaun Collin* - The Department of Anatomy, University of Queensland, Australia.

These samples are for Shaun to follow up on previous investigations in the anatomy of the retina in deep-sea fish: *Scopelarchus analis* (4), *Opisthoproctus soleus* (2), *Stylephorus chordatus* (3), *Anoplogaster cornuta* (3), *Gigantura chuni* (2), *Histeoteuthis* sp (3), *Dolichopteryx longipes* (1), *Winteria* sp. (1), *Alepocephalus spp.* (3).

*Ellis Loew* - The Department of Lots of Stuff, Cornell University, USA.

Liver and muscle tissue from 6 species of benthic (3-4000m) fish and whole bodies of 20 species of mesopelagic fish have been snap frozen for Ellis. His is interested in the properties of fatty tissue under pressure.

Good collaborative links have been forged with Jochen Wagner, David Hunt, Edie Widder, Monty Priede and Sue Way during the cruise.

## Funding and Finances Associated with Cruise

No specific funding or grants were applied for. As this was a relatively low cost exercise for me, enough funding was available from my Australian Research Council QEII Fellowship grant and ARC Large Research grant, both of which had provision for a small amount of deep-sea work. My budget was as follows:

| | |
|---|---|
| Travel and subsistence | $ 2500 Au |
| Consumables | $ 1000 Au |
| Use of EM facility | $ 1500 Au |
| [Total *ca* | £ 2000 GBP] |

**Justin Marshall**

### 9.3.15 Video and Digital Imaging of Deep-Sea Animals

Intensified video recordings of bioluminescence were made from:

*Borostomias elucens*
*Cryptopsaras couesi*
*Malacosteus niger*
*Pachystomias microdon*
*Photostomias guernei*
*Searsia koefoedi*

These recordings will be analyzed for emission kinetics.

A small tissue sample (5 suckers) was taken from the large Cirrate octopod (*Cirroeuthis* sp.) collected by OTSB on Nov. 12. This will be sent to Dr. Elizabeth Balser at Illinois Wesslyan for comparison with suckers taken from the recently described bioluminescent suckers of *Stauroteuthis syrtensis*.

Using a high resolution digital camera (2000 X 3000 pixels) 862 images of trawl caught specimens were recorded. These included:

| **Fish:** | **Invertebrates:** |
|---|---|
| *Anoplogaster cornuta* | *Abraliopsis sp.* |
| *Argyropelecus affinis* | *Argonauta sp.* |
| *Argyropelecus gigas* | *Bathethauma lyromma* |
| *Argyropelecus olfersi* | *Chiroteuthis sp.* |
| *Aristostomias grimaldii* | *Cirroteuthis sp.* |
| Astronesthid | *Conchoecia sp.* |
| *Bathophilus nigerrimus* | *Cranchia scabra* |
| *Bathylychnops exilis* | *Cystisoma sp.* |
| *Bonapartia pedaliota* | *Disseta palumboi* |
| *Borostomias elucens* | *Eledonella pygmaea* |
| *Caulophryne sp.* | *Eupasiphaea gilesi* |
| *Cetomimus sp.* | *Gaussia princeps* |
| *Chaenophryne draco* | *Gigantocypris mulleri* |
| *Charapicthys sp.* | *Gnathophausia ingens* |
| *Chauliodus danae* | *Grimalditeuthis bomplandii* |
| *Chiasmodon bolangeri* | *Hippopodius sp.* |
| *Caristius maderensis* | *Histioteuthis sp.* |
| *Cryptopsaras couesi* | *Japetella diaphana* |
| *Cyclothone pallida* | *Lanceola sp.* |
| *Diaphus sp.* | *Liocranchia sp.* |
| *Diplophos taenia* | *Macrocypridina sp.* |
| *Eurypharynx pelecanoides* | *Mastigoteuthis sp.* |
| *Flagellostomias boureei* | Nemertine |
| *Gonostoma elongatum* | *Notostomus sp.* |
| *Himantolophus sp.* | Octopus |
| *Hymenocephalus italicus* | *Onychoteuthis banksi* |
| *Lampadena urophaos atlantica* | *Pasiphaea hoplocerca* |
| *Lampanyctus alatus* | *Pegohyperia sp.* |
| *Lampanyctus intricarius* | *Phronima sp.* |
| *Lamprogrammus niger* | *Phronima w/ babies* |
| Leptocephalus larva | Phyllosoma larva |
| *Linophryne coronata* | Physonect siphonophore |
| *Malacosteus niger* | *Plesionika sp.* |
| *Melanocetus johnsonii* | *Plutonaster sp.* |
| *Melanonus zugmayeri* | *Pyrosoma atlanticum* |
| Melanostomid | *Pyroteuthis sp.* |
| *Myctophum selenops* | *Scypholanceola sp.* |

| | |
|---|---|
| *Normichthys operosus* | *Streetsia sp.* |
| *Omusudis lowei* | *Teuthowenia megalops* |
| *Oneirodes eschrichtii* | Tomopterid |
| *Oneirodes sp.* | |
| *Opisthoproctus soleatus* | |
| *Pachystomias microdon* | |
| *Photostomias guernei* | |
| *Photostylus pycnopterus* | |
| *Platytroctes apus* | |
| *Psednos sp.* | |
| *Rhynchohyalus natalensis* | |
| *Saccopharynx sp.* | |
| *Scopelarchus analis* | |
| *Scopelogadus beani* | |
| *Searsia koefoedi* | |
| *Serrivomer beani* | |
| *Serrivomer brevidentatus* | |
| *Sternoptyx sp.* | |
| *Stomias boa* | |
| *Stomias brevibarbatus* | |
| *Stomias sp.* | |
| *Stylephorus chordatus* | |
| Unidentified fish larva | |
| *Vinciguerria sp.* | |
| *Winteria telescopa* | |

Many of these images will be used in a book that I am writing as an introduction to marine bioluminescence. Some of the images will also be used on our laboratory web site, which provides educational information about marine bioluminescence.

A CD ROM of selected images will be made available to cruise participants for teaching purposes.

Cruise costs: Estimated total costs for cruise participation including overnight accommodations in Tenerife and supplies: $7,500. These funds came from internal funding, primarily generated by the sale of images and video recordings.

**Edie Widder**

### 9.3.16 Natural History Film Production

**Summary of Cruise Objectives**

As representatives from the BBC's Natural History Unit, we participated in the *Discovery* 243 cruise with the primary objective of obtaining unique new video footage of deep sea animals for inclusion in a major new documentary series on the natural history of the world's oceans. This series, to be titled '*The Blue Planet*', will comprise eight fifty-minute programmes, encompassing a variety of global marine habitats, such as coral reefs, cold temperate seas, the open ocean and the deep sea. Each programme will focus on the biology and behaviour of the animals that inhabit such marine environments, aiming to both educate and entertain the viewer. Filmed on the latest widescreen digital technology, the series will transmit world-wide in Autumn 2001.

For the final programme of the series - The Deep Sea - it was clear that we would have to film much of the material with animals brought to the surface in nets, due to the inherent technical difficulties, expense and unpredictability of finding and filming

deep sea fish in their natural habitat. The *Discovery* cruise 243 comprised a number of vital elements for helping us to achieve this goal.

One of the key challenges to overcome when filming deep sea animals that have been brought up in nets is that of obtaining specimens in good condition. It was our hope that the use of the closing cod-end, as operated on the RMT 1+8 net system on *Discovery*, would provide a unique opportunity to sample deep sea fish and other animals and bring them up intact and, in some cases, alive. Prior to the cruise, we had identified a number of key species for which the *Discovery* 243 cruise was our only opportunity for collection and filming - these included gulper eels, several species of angler fish, dragon fish and shrimp.

## Results & Future Plans

The mid-water trawls carried out during the course of the Discovery 243 cruise sampled a collection of deep sea animals which exceeded our expectations, both in terms of number and variety of animals caught and also in the high quality of their condition. A summary of species filmed for '*The Blue Planet*' series is as follows:

*Gigantocypris* - we were able to obtain a variety of shots of several animals free-swimming in a kreisel tank, as well as close-ups showing detail of this creature's huge eyes.
*Gnathophausia* - a spectacular red mysid shrimp in perfect condition, filmed swimming actively in the kreisel.
*Systellaspis* - detailed filming on eye movement.
*Carinaria* - very exciting predation on a small *Stomias* fish.
Angler fish - perhaps the biggest success story of this shoot. *Melanocetus* were consistently caught in such good condition that we were able to film these anglers swimming well in the kreisel, as well as doing close-ups in a smaller tank. We were also able to film a variety of other angler fish species in order to highlight details on lure design, and sensory mechanisms - a live *Himantolophus* covered in rivet-like pores (which also waved its lure back and forth) and a *Caulophryne* with a mass of hairy projections. Perhaps most exciting of all, a large female *Melanocetus* was caught with a parasitic male attached - an extremely rare observation providing totally unique footage for *The Blue Planet* series.
Live *Anoplogaster* - a very active fish, filmed swimming well in the kreisel.
*Opisthoproctus* - detail on eyes.
*Winteria* - another unexpected surprise. This very rare and unusual-looking fish was caught alive and in immaculate condition, allowing us to obtain shots of it swimming in a tank, with details on its huge tube eyes.
Live *Stomias* and *Photostomias* - various shots of fish in good condition.
Two good *Malacosteus* - various shots.
*Poromitra* - mostly detail of pores on specialised scales for detecting movement, plus a couple of shots of swimming in kreisel.
A variety of transparent octopods and squid in kreisel.
Gulper eels - *Eupharynx* and *Saccopharynx*. Although these fish always came up dead, we were able to film a number of aspects of the shape of the animal, its jaw movement and details on the lateral line, which will most likely be used to recreate an animation sequence using computer graphics.

All video material gathered on the *Discovery* 243 cruise will be returned to the Natural History Unit in Bristol and will be logged, ready for the programme edit in September 2000. VHS copies of any material of interest will be made available to participating scientists, for personal and educational purposes only.

## Summary and Acknowledgements

The scientific work carried out on *Discovery* 243 provided the BBC Natural History Unit with a unique and exciting opportunity to see and film an array of fascinating

deep sea fish and other animals. We are extremely grateful to all those who so generously allowed us to whisk away their specimens for filming and were privileged to be in the company of so many deep sea biology experts whose knowledge, enthusiasm and patience knew no bounds. The cruise has provided us with many hours of footage, some of it showing species and behaviours rarely captured on film before. Obviously, whilst we are delighted with the successes that we achieved once the winch had been repaired, we will never know what was missed during the days of down-time.

**Penny Allen & David Shale**

## 10. D243 STATION LIST

**Notes:**

1. Latitudes and Longitudes as well as times may differ from those noted in the narrative of Section @@@ because in this list they refer to times and places where the deployed gear was active (e.g. nets open and fishing), rather than when gear entered the water. Similarly, tese data may differ slightly from information given in Section X@.2 (Landers) because of, for instance, minor discrepancies in time between when a lander is deployed and when its dahn buoy sinks.
2. RMT1/RMT8 refers to the RMT1+8 midwater net
3. RMT1/RMT8/CCE refers to the RMT1+8CCE midwater net. This was fished with a scope following the formula between meters of wire out(w), and depth (d) as: d=0.5w+60
4. RMT25/4.5 refers to the RMT25 midwater net. This was fished with a scope of 2:1
5. OTSB14 refers to the semi balloon benthic otter trawl. This was fished with a scope of 3:1
5. Fishing depths are recorded from the surface (!) with a zero in parenthesis, followed by the depth range over which the fishing took place. In the case of the RMT1+8 or RMT1+8CCE these are the depths recorded in the fishing log from the acoustic transducer on the net. In the case of the RMT25 these depths are estimates based on knowledge of the nets scope (m.w.o. to depth ratio).
6. In this list all stations have a “#xxx” suffix indicating a series number, even when only one activity occurred at that station. During the cruise such suffixes were only used when an uniterupted series of deployments were made using the same gear.

| Station | Date | Lat | Long | Gear | Depth | Fishing time | Flow Dist | comment |
|---|---|---|---|---|---|---|---|---|
| Series | 1999 | (Start/Finish) | | | | | | |
| | | deg min | deg min | | (M) | (GMT) | (kM) | |
| 13630#001 | 14.x | 44 8.5N | 10 43.3W | ISIT | 0 - 4939 | 1248-1248 | | Test deployment of ISIT |
| | | 44 8.5N | 10 43.3W | | -( 0) | | | lander |
| 13631#001 | 16.x | 36 54.9N | 14 30.1W | RMT1 | ( 0)- 636 | 1003-1205 | | Materials haul |
| | | | | RMT8 | | | | |
| | | 36 49.7N | 14 31.3W | CCE | - 788 | | | |
| 13632#001 | 19.x | 26 50.4N | 18 12.4W | ISIT | ( 0)- 3730 | 1603 | - | |
| | 20.x | 26 40.1N | 18 8.2W | | - 3730 | - | 0000 | |
| 13633#001 | 20.x | 26 53.2N | 18 13.4W | RMT1 | ( 0)- 617 | 0923-1044 | | Materials haul |
| | | | | RMT8 | | | | |
| | | 26 55.3N | 18 14.8W | CCE | - 1000 | | | |
| 13634#001 | 20.x | 27 16.8N | 17 44.6W | ISIT | ( 0)- 3690 | 1945 | - | |
| | 21.x | 27 18.4N | 17 47.5W | | - 3690 | - | 0000 | |
| 13635#001 | 21.x | 27 43.6N | 16 59.0W | AUDOS | ( 0)- 3351 | 1855 | - | |
| | 22.x | 27 43.4N | 16 58.8W | | - 3351 | - | 0000 | |
| 13636#001 | 23.x | 28 43.0N | 15 44.6W | RMT1 | ( 0)- 520 | 1956-2057 | | Materials haul |
| | | | | RMT8 | | | | |
| | | 28 44.8N | 15 45.5W | CCE | - 670 | | | |
| 13637#001 | 24.x | 28 47.9N | 15 45.0W | RMT1 | ( 0)- 890 | 1911-2011 | | Materials haul |
| | | | | RMT8 | | | | |
| | | 28 48.8N | 15 46.7W | CCE | - 1004 | | | |
| 13638#001 | 26.x | 26 57.6N | 16 45.2W | RMT1 | ( 0)- 800 | 0132-0333 | | Materials haul |
| | | 27 1.4N | 16 47.3W | RMT8 | - 1020 | | | |
| 13638#002 | 26.x | 27 4.9N | 16 49.9W | RMT1 | ( 0)- 955 | 0553-0800 | | Materials haul |
| | | 27 8.6N | 16 53.7W | RMT8 | - 1192 | | | |
| 13638#003 | 26.x | 27 13.8N | 16 57.7W | RMT1 | ( 0)- 1010 | 1048-1148 | | Materials haul |
| | | 27 15.7N | 16 58.9W | RMT8 | - 1200 | | | |

| Station | Date | Lat | Long | Gear | Depth | Fishing Flow | comment |
|---|---|---|---|---|---|---|---|
| Series | 1999 | (Start/Finish) | | | | time | Dist |
| | | deg min | deg min | | (M) | (GMT) | (kM) |
| 13639#001 | 28.x | 17 40.8N | 20 0.2W | AUDOS | ( 0)- 3254 | 2048 | - |
| | 29.x | 17 46.1N | 19 59.2W | | - 3254 | - 0000 | |
| 13640#001 | 28.x | 17 41.4N | 20 0.1W | ISIT | ( 0)- 3251 | 2112 | - |
| | 29.x | 17 46.1N | 19 59.2W | | - 3251 | - 0000 | |
| 13641#001 | 28.x | 17 43.1N | 19 59.8W | RMT1 | ( 0)- 800 | 2229 | - Materials haul |
| | | | | RMT8 | | | |
| | 29.x | 17 47.0N | 19 58.6W | CCE | - 1000 | - 0029 | |
| 13641#002 | 29.x | 17 50.3N | 19 56.7W | RMT1 | ( 0)- 483 | 0227-0427 | Materials haul |
| | | | | RMT8 | | | |
| | | 17 53.9N | 19 54.3W | CCE | - 580 | | |
| 13641#003 | 29.x | 17 44.6N | 19 58.4W | RMT1 | ( 0)- 400 | 1047-1147 | Materials haul |
| | | | | RMT8 | | | |
| | | 17 46.7N | 19 57.1W | CCE | - 535 | | |
| 13641#004 | 29.x | 17 50.6N | 19 54.6W | RMT1 | ( 0)- 1030 | 1357-1500 | Materials haul |
| | | | | RMT8 | | | |
| | | 17 52.8N | 19 53.3W | CCE | - 1200 | | |
| 13642#001 | 29.x | 17 40.2N | 20 0.4W | ISIT | ( 0)- 3256 | 1908 | - |
| | 30.x | 17 42.4N | 20 2.0W | | - 3256 | - 0000 | |
| 13643#001 | 29.x | 17 36.5N | 20 4.6W | RMT1 | ( 0)- 215 | 2028-2158 | Materials haul |
| | | | | RMT8 | | | |
| | | 17 39.3N | 20 3.4W | CCE | - 328 | | |
| 13643#002 | 30.x | 17 43.4N | 20 1.6W | RMT1 | ( 0)- 1480 | 0029-0259 | Materials haul |
| | | | | RMT8 | | | |
| | | 17 47.9N | 19 58.2W | CCE | - 1700 | | |
| 13643#003 | 30.x | 17 42.6N | 20 0.0W | RMT1 | ( 0)- 718 | 1116-1316 | Materials haul |
| | | | | RMT8 | | | |
| | | 17 46.6N | 19 57.8W | CCE | - 800 | | |

| Station | Date | Lat | Long | Gear | Depth | Fishing | Flow | comment |
|---|---|---|---|---|---|---|---|---|
| Series | 1999 | (Start/Finish) | | | | time | Dist | |
| | | deg min | deg min | | (M) | (GMT) | (kM) | |
| 13644#001 | 30.x | 17 30.2N | 20 14.9W | AUDOS | ( 0)- 3244 | 1735 | - | |
| | 31.x | 17 36.8N | 20 10.1W | | - 3244 | - | 0000 | |
| 13645#001 | 30.x | 17 25.8N | 20 17.0W | RMT1 | ( 0)- 400 | 1910-2046 | Materials haul | |
| | | | | RMT8 | | | | |
| | | 17 29.6N | 20 15.1W | CCE | - 500 | | | |
| 13645#002 | 30.x | 17 34.1N | 20 12.2W | RMT1 | ( 0)- 220 | 2249-2349 | Materials haul | |
| | | | | RMT8 | | | | |
| | | 17 36.4N | 20 10.5W | CCE | - 302 | | | |
| 13645#003 | 31.x | 17 39.8N | 20 7.3W | RMT1 | ( 0)- 500 | 0132-0305 | Materials haul | |
| | | | | RMT8 | | | | |
| | | 17 42.9N | 20 4.3W | CCE | - 615 | | | |
| 13646#001 | 1.xi | 15 0.0N | 20 29.9W | AUDOS | ( 0)- 4046 | 0724 | - | |
| | 2.xi | 15 13.3N | 20 20.3W | | - 4046 | - | 0000 | |
| 13647#001 | 1.xi | 14 55.7N | 20 31.5W | RMT25/4.5 | ( 0)- 950 | 0854-1410 | Materials haul | |
| | | 15 7.0N | 20 26.9W | | - 950 | | | |
| 13647#002 | 1.xi | 15 7.4N | 20 26.8W | RMT25/4.5 | ( 0)- 1200 | 1420-1952 | Materials haul | |
| | | 15 17.8N | 20 19.0W | | - 1200 | | | |
| 13647#003 | 1.xi | 15 10.1N | 20 22.5W | RMT25/4.5 | ( 0)- 300 | 2136 | - | Materials haul |
| | 2.xi | 15 13.3N | 20 20.2W | | - 300 | - | 0007 | |
| 13647#004 | 2.xi | 15 13.5N | 20 19.9W | RMT25/4.5 | ( 0)- 600 | 0026-0340 | Materials haul | |
| | | 15 16.9N | 20 17.8W | | - 600 | | | |
| 13647#005 | 2.xi | 14 54.3N | 20 31.3W | RMT25/4.5 | ( 0)- 2000 | 0915-1715 | Materials haul | |
| | | 15 5.4N | 20 28.9W | | - 2000 | | | |

| Station | Date | Lat | Long | Gear | Depth | Fishing | Flow | comment |
|---|---|---|---|---|---|---|---|---|
| Series | 1999 | (Start/Finish) | | | | time | Dist | |
| | | deg min | deg min | | (M) | (GMT) | (kM) | |
| 13648#001 | 2.xi | 15 0.1N | 20 30.0W | ISIT | ( 0)- 4046 | 1841 | - | |
| | 3.xi | 15 6.2N | 20 30.2W | | - 4046 | - | 0000 | |
| 13649#001 | 2.xi | 14 57.8N | 20 31.2W | RMT1 | ( 0)- 485 | 1956-2126 | Materials haul | |
| | | | | RMT8 | | | | |
| | | 15 0.9N | 20 30.9W | CCE | - 624 | | | |
| 13649#002 | 2.xi | 15 3.7N | 20 30.5W | RMT1 | ( 0)- 401 | 2253 | - | Materials haul |
| | | | | RMT8 | | | | |
| | 3.xi | 15 7.0N | 20 30.1W | CCE | - 498 | - | 0023 | |
| 13649#003 | 3.xi | 15 9.2N | 20 29.9W | RMT1 | ( 0)- 10 | 0140-0310 | Materials haul | |
| | | | | RMT8 | | | | |
| | | 15 12.0N | 20 30.0W | CCE | - 300 | | | |
| 13650#001 | 3.xi | 15 1.7N | 20 30.0W | AUDOS | ( 0)- 4038 | 0921 | - | |
| | 4.xi | 15 15.9N | 20 24.3W | | - 4038 | - | 0000 | |
| 13651#001 | 3.xi | 14 58.6N | 20 29.3W | RMT25/4.5 | ( 0)- 1200 | 1005-1545 | Materials haul | |
| | | 15 8.5N | 20 27.0W | | - 1200 | | | |
| 13651#002 | 3.xi | 15 8.5N | 20 26.9W | RMT25/4.5 | ( 0)- 200 | 1555-1710 | Materials haul | |
| | | 15 10.2N | 20 26.2W | | - 200 | | | |
| 13651#003 | 3.xi | 15 10.4N | 20 26.2W | RMT25/4.5 | ( 0)- 700 | 1720-2115 | Materials haul | |
| | | 15 18.0N | 20 25.3W | | - 700 | | | |
| 13652#001 | 3.xi | 15 14.3N | 20 25.2W | RMT1 | ( 0)- 500 | 2255 | - | Materials haul |
| | | | | RMT8 | | | | |
| | 4.xi | 15 16.6N | 20 23.9W | CCE | - 700 | - | 0025 | |
| 13652#002 | 4.xi | 15 16.6N | 20 23.6W | RMT1 | ( 0)- 50 | 0214-0344 | Materials haul | |
| | | | | RMT8 | | | | |
| | | 15 18.5N | 20 23.9W | CCE | - 300 | | | |

| Station | Date | Lat | Long | Gear | Depth | Fishing Flow comment |
|---|---|---|---|---|---|---|
| Series | 1999 | (Start/Finish) | | | | time Dist |
| | | deg min | deg min | | (M) | (GMT) (kM) |
| 13652#003 | 4.xi | 14 57.9N | 20 30.0W | RMT1 | ( 0)- 1010 | 1010-1110 Materials haul |
| | | | | RMT8 | | |
| | | 14 59.8N | 20 29.7W | CCE | - 1200 | |
| 13653#001 | 4.xi | 14 58.9N | 20 29.8W | ISIT | ( 0)- 3845 | 1329-1729 |
| | | 14 58.5N | 20 29.8W | | - 3845 | |
| 13654#001 | 4.xi | 14 58.6N | 20 30.0W | AUDOS | ( 0)- 4046 | 1802 - |
| | 5.xi | 15 4.5N | 20 28.1W | | - 4046 | - 0000 |
| 13655#001 | 4.xi | 14 58.4N | 20 29.7W | RMT1 | ( 0)- 800 | 2023-2153 Materials haul |
| | | | | RMT8 | | |
| | | 15 1.0N | 20 29.1W | CCE | - 1000 | |
| 13655#002 | 4.xi | 15 3.5N | 20 28.4W | RMT1 | ( 0)- 52 | 2321 - Materials haul |
| | | | | RMT8 | | |
| | 5.xi | 15 5.9N | 20 27.8W | CCE | - 307 | - 0051 |
| 13655#003 | 5.xi | 15 7.7N | 20 27.3W | RMT1 | ( 0)- 530 | 0212-0342 Materials haul |
| | | | | RMT8 | | |
| | | 15 10.7N | 20 26.4W | CCE | - 700 | |
| 13655#004 | 5.xi | 15 14.9N | 20 25.6W | RMT1 | ( 0)- 1025 | 0557-0757 Materials haul |
| | | | | RMT8 | | |
| | | 15 19.6N | 20 24.7W | CCE | - 1274 | |
| 13656#001 | 5.xi | 14 59.8N | 20 29.6W | ISIT | ( 0)- 4045 | 1714-1714 |
| | | 14 59.8N | 20 29.6W | | - 4045 | |
| 13657#001 | 5.xi | 14 57.5N | 20 30.0W | RMT25/4.5 | ( 0)- 1000 | 1745-2310 Materials haul |
| | | 15 5.9N | 20 27.9W | | - 1000 | |
| 13657#002 | 5.xi | 15 6.2N | 20 27.9W | RMT25/4.5 | ( 0)- 700 | 2327 - Materials haul |
| | 6.xi | 15 11.1N | 20 27.7W | | - 700 | - 0320 |

| Station | Date | Lat | Long | Gear | Depth | Fishing | Flow | comment |
|---|---|---|---|---|---|---|---|---|
| Series | 1999 | (Start/Finish) | | | | time | Dist | |
| | | deg min | deg min | | (M) | (GMT) | (kM) | |
| 13657#003 | 6.xi | 14 54.9N | 20 30.2W | RMT25/4.5 | ( 0)- 1200 | 0835-1545 | | Materials haul |
| | | 15 3.5N | 20 26.6W | | - 1200 | | | |
| 13658#001 | 6.xi | 15 0.1N | 20 30.0W | ISIT | ( 0)- 4046 | 1630-1630 | | |
| | | 15 0.1N | 20 30.0W | | - 4046 | | | |
| 13659#001 | 6.xi | 14 59.1N | 20 30.2W | RMT1 | ( 0)- 700 | 1740-1910 | | Materials haul |
| | | | | RMT8 | | | | |
| | | 15 2.0N | 20 28.7W | CCE | - 826 | | | |
| 13659#002 | 6.xi | 15 4.7N | 20 26.8W | RMT1 | ( 0)- 300 | 2100-2230 | | Materials haul |
| | | | | RMT8 | | | | |
| | | 15 7.4N | 20 25.5W | CCE | - 603 | | | |
| 13659#003 | 6.xi | 15 8.9N | 20 24.9W | RMT1 | ( 0)- 201 | 2335 - | | Materials haul |
| | | | | RMT8 | | | | |
| | 7.xi | 15 11.0N | 20 24.1W | CCE | - 401 | - 0105 | | |
| 13659#004 | 7.xi | 15 12.3N | 20 23.5W | RMT1 | ( 0)- 50 | 0200-0330 | | Materials haul |
| | | | | RMT8 | | | | |
| | | 15 14.7N | 20 22.7W | CCE | - 300 | | | |
| 13660#001 | 7.xi | 15 0.9N | 20 30.2W | RMT25/4.5 | ( 0)- 800 | 0830-1320 | | Materials haul |
| | | 15 5.8N | 20 29.6W | | - 800 | | | |
| 13660#002 | 7.xi | 15 5.9N | 20 29.6W | RMT25/4.5 | ( 0)- 500 | 1325-1700 | | Materials haul |
| | | 15 8.7N | 20 29.5W | | - 500 | | | |
| 13661#001 | 7.xi | 15 0.0N | 20 30.1W | ISIT | ( 0)- 4048 | 1827 - | | |
| | 8.xi | 14 41.9N | 20 29.6W | | - 4048 | - 0000 | | |
| 13662#001 | 8.xi | 14 46.6N | 20 28.7W | OTSB14 | ( 0)- 4051 | 0215-0320 | | Materials haul |
| | | 14 49.6N | 20 28.2W | | - 4074 | | | |
| 13662#002 | 8.xi | 14 57.7N | 20 27.5W | OTSB14 | ( 0)- 4031 | 1844-2315 | | Materials haul |
| | | 15 5.9N | 20 35.6W | | - 4052 | | | |

| Station | Date | Lat | Long | Gear | Depth | Fishing | Flow | comment |
|---|---|---|---|---|---|---|---|---|
| Series | 1999 | (Start/Finish) | | | | time | Dist | |
| | | deg min | deg min | | (M) | (GMT) | (kM) | |
| 13663#001 | 9.xi | 17 40.0N | 20 0.0W | AUDOS | ( 0)- 3258 | 1915 | - | |
| | 10.xi | 17 47.6N | 19 58.5W | | - 3259 | - | 0000 | |
| 13664#001 | 10.xi | 17 51.4N | 19 56.6W | OTSB14 | ( 0)- 3219 | 0140-0425 | Materials haul | |
| | | 17 58.1N | 19 56.7W | | - 3232 | | | |
| 13665#001 | 10.xi | 17 40.9N | 20 0.1W | RMT25/4.5 | ( 0)- 1000 | 1330-1935 | Materials haul | |
| | | 17 52.2N | 19 56.5W | | - 1000 | | | |
| 13666#001 | 10.xi | 17 53.0N | 19 56.3W | AUDOS | ( 0)- 3220 | 2036 | - | |
| | 11.xi | 17 55.6N | 19 56.3W | | - 3220 | - | 0000 | |
| 13667#001 | 10.xi | 17 51.1N | 19 57.2W | RMT1 | ( 0)- 561 | 2153-2323 | Materials haul | |
| | | | | RMT8 | | | | |
| | | 17 54.2N | 19 56.5W | CCE | - 640 | | | |
| 13667#002 | 11.xi | 17 56.8N | 19 56.1W | RMT1 | ( 0)- 50 | 0040-0210 | Materials haul | |
| | | | | RMT8 | | | | |
| | | 17 59.6N | 19 55.5W | CCE | - 300 | | | |
| 13667#003 | 11.xi | 18 1.0N | 19 55.3W | RMT1 | ( 0)- 50 | 0300-0430 | Materials haul | |
| | | | | RMT8 | | | | |
| | | 18 4.0N | 19 54.7W | CCE | - 300 | | | |
| 13668#001 | 11.xi | 17 49.6N | 19 56.9W | AUDOS | ( 0)- 3198 | 1343 | - | |
| | 12.xi | 17 54.2N | 19 56.0W | | - 3198 | - | 0000 | |
| 13669#001 | 11.xi | 17 51.5N | 19 56.4W | RMT1 | ( 0)- 850 | 1500-1600 | Materials haul | |
| | | | | RMT8 | | | | |
| | | 17 53.4N | 19 55.7W | CCE | - 953 | | | |
| 13670#001 | 11.xi | 17 43.2N | 19 57.7W | RMT25/4.5 | ( 0)- 1300 | 1910 | - | Materials haul |
| | 12.xi | 17 58.6N | 19 52.5W | | - 1300 | - | 0320 | |
| 13671#001 | 12.xi | 17 49.9N | 20 3.3W | AUDOS | ( 0)- 3208 | 0930 | - | |
| | 13.xi | 18 7.0N | 19 53.5W | | - 3208 | - | 0000 | |

| Station | Date | Lat | Long | Gear | Depth | Fishing | Flow | comment |
|---|---|---|---|---|---|---|---|---|
| Series | 1999 | (Start/Finish) | | | | time | Dist | |
| | | deg min | deg min | | (M) | (GMT) | (kM) | |
| 13672#001 | 12.xi | 17 56.6N | 19 56.8W | OTSB14 | ( 0)- 3238 | 1331-1753 | | Materials haul |
| | | 18 6.0N | 19 51.4W | | - 3255 | | | |
| 13673#001 | 12.xi | 18 5.4N | 19 53.8W | RMT1 | ( 0)- 406 | 2321 | - | Materials haul |
| | | | | RMT8 | | | | |
| | 13.xi | 18 8.8N | 19 52.8W | CCE | - 598 | - 0051 | | |
| 13673#002 | 13.xi | 18 11.0N | 19 51.8W | RMT1 | ( 0)- 72 | 0204-0304 | | Materials haul |
| | | | | RMT8 | | | | |
| | | 18 13.1N | 19 51.1W | CCE | - 302 | | | |
| 13674#001 | 13.xi | 17 51.8N | 20 3.5W | ISIT | ( 0)- 3201 | 0924 | | |
| | | 00 00.00 | 000 00.0W | | - 3201 | | | |
| 13675#001 | 13.xi | 17 51.0N | 20 4.7W | RMT25/4.5 | ( 0)- 1500 | 1000-1810 | | Materials haul |
| | | 18 3.0N | 19 58.8W | | - 1500 | | | |
| 13675#002 | 13.xi | 18 3.1N | 19 58.7W | RMT25/4.5 | ( 0)- 700 | 1815-2249 | | Materials haul |
| | | 18 11.3N | 19 53.7W | | - 700 | | | |
| 13676#001 | 14.xi | 17 54.0N | 20 3.8W | AUDOS | ( 0)- 3199 | 0925 | | |
| | | 00 00.00 | 000 00.0W | | - 3199 | | | |
| 13677#001 | 14.xi | 17 53.4N | 20 4.6W | RMT25/4.5 | ( 0)- 1200 | 1000-1655 | | Materials haul |
| | | 18 4.0N | 20 0.1W | | - 1200 | | | |
| 13677#002 | 14.xi | 18 4.0N | 20 0.1W | RMT25/4.5 | ( 0)- 1000 | 1655-2255 | | Materials haul |
| | | 18 13.7N | 19 55.3W | | - 1000 | | | |
| 13677#003 | 14.xi | 18 13.8N | 19 55.2W | RMT25/4.5 | ( 0)- 300 | 2300 | - | Materials haul |
| | 15.xi | 18 16.7N | 19 52.3W | | - 300 | - 0121 | | |
| 13678#001 | 15.xi | 17 57.3N | 20 3.1W | ISIT | ( 0)- 3199 | 0927 | | |
| | | 00 00.00 | 000 00.0W | | - 3199 | | | |
| 13679#001 | 15.xi | 17 59.2N | 20 2.6W | RMT1 | ( 0)- 550 | 1025-1155 | | Materials haul |
| | | | | RMT8 | | | | |
| | | 18 2.3N | 20 1.3W | CCE | - 640 | | | |

## 11. TRACK CHART